Acetylcholinesterase activity as a biomarker of exposure to antibiotics and pesticides in the black tiger shrimp (Penaeus monodon)

Huynh Thi Tu a,b,*, Frederic Silvestre a, Marie-Louise Scippo c, Jean-Pierre Thome d, Nguyen Thanh Phuong b, Patrick Kestemont a

a Unit of Research in Organismal Biology, University of Namur, 61, rue de Bruxelles, B-5000 Namur, Belgium
b College of Aquaculture and Fisheries, University of Can Tho, 3/2 Street, Campus II, Can Tho City, Vietnam
c Centre of Analysis of Residues in Traces, University of Liege, B43b, Sart-Tilman, B-4000 Liege, Belgium
d Laboratory of Animal Ecology and Ecotoxicology, University of Liege, Allée du 6 aout, B-4000 Liege, Belgium

Abstract

This study aimed to assess the potentiality to use cholinesterase activity (ChE) in black tiger shrimp (Penaeus monodon) as a biomarker of exposure to 2 antibiotics (enrofloxacin, furazolidone) and 2 pesticides (endosulfan, deltamethrin), commonly used in Vietnamese farms. ChE from muscle and gills was first characterised using three different substrates and specific inhibitors. Results showed that both tissues possess only one ChE which displays the typical properties of an acetylcholinesterase (AChE). In a second part, shrimp (average weight of 8.8–10 g) were fed with medicated-feed containing 4 g enrofloxacin (quinolone) or furazolidone (nitrofuran)/kg for 7 days, or exposed to 3 actual concentrations of endosulfan (0, 0.009, 0.09, 0.9 m) or deltamethrin (0, 0.0007, 0.007, 0.07 μg/L) for 4 days. After treatment, animals were decontaminated during 7 days. We observed that AChE activity in muscle was not significantly affected in shrimp fed with enrofloxacin or furazolidone, while it significantly decreased (up to 28%) in gills of shrimp fed with furazolidone. Following endosulfan and deltamethrin exposure, no significant changes in AChE activity were observed in gills. However, a significant decrease occurred in muscle after 4 days exposure (inhibition of 30% and 49% at 0.9 μg/L endosulfan and 0.07 μg/L deltamethrin, respectively). While muscle AChE activity should be assessed to point out endosulfan or deltamethrin exposure, gill AChE activity impairment could indicate an exposure to furazolidone. The present study underlines the benefits to use AChE as a biomarker of chemotherapeutics as part of an integrated aquaculture management to reach industry sustainability.

1. Introduction

Black tiger shrimp (Penaeus monodon) (Fabricius, 1798) aquaculture has been developing rapidly in Vietnam in recent years. Shrimp farming growth has led to increased use of drugs and chemicals for prevention and for treatment of infectious diseases. Among them, antibiotics such as enrofloxacin and pesticides such as endosulfan and deltamethrin have been and are still the most commonly used (Tu et al., 2006). Although the use of furazolidone was prohibited in 1995, it is still used occasionally in shrimp aquaculture (Tu et al., 2006). There is an increasing awareness that exposure to chemotherapeutics could slow down growth, favour diverse pathologies, increase cultured shrimp mortality and increase the risk for consumers as well as have impacts on adjacent ecosystems (Graslund et al., 2003). Thus, there is a need for fast and sensitive methods to assess the toxicological effects of drugs and chemicals on cultured shrimp.

Enrofloxacin (quinolone) has been used extensively to treat systemic bacterial infections in fish (Scallan and Smith, 1985). The pharmacokinetics of this antibiotic have been studied in Chinese shrimp (Penaeus chinensis) (Xu et al., 2006), Chinese mitten crab (Eriocheir sinensis) (Tang et al., 2006), whiteleg shrimp (Litopenaeus vannamei) (Fang et al., 2004) and Chinese white shrimp (Fenneropenaeus chinensis) (Fang et al., 2004). Since an excess amount of enrofloxacin is harmful to people, the European Union set a maximum residue limit of 100 μg/kg for enrofloxacin and its metabolite ciprofloxacin, in the edible tissues of aquatic animals (Commission Regulation, EEC No. 1181/2002). Furazolidone (nitrofuran) has shown carcinogenic and mutagenic characteristics (Auro et al., 2004). This has led to a prohibition of furazolidone for the treatment of animals used for food production in Europe since 1995 (Commission Regulation, EEC No. 1442/1995). The pharmacokinetics of furazolidone have been examined...
Nile tilapia (*Oreochromis niloticus*) (Xu et al., 2006). In Vietnam, these antibiotics are used during the culture of shrimp, mainly to prevent (prophylactic use) and treat (therapeutic use) bacterial diseases. Enrofloxacin and furazolidone are generally blended with feed at dose of 4–5 g/kg feed for 7 days (Tu et al., 2006). The usage of antibiotics has brought about great concern. The unconsumed food and shrimp faeces containing antibiotics reach the sediment or can be washed by currents to distant sites. Once in the environment, these antibiotics can be ingested by wild fish, shrimp and other organisms. These residual antibiotics can remain in the sediment, exerting selective pressure, thereby altering the composition of its microflora and selecting for antibiotic-resistant bacteria. The determinants of antibiotic resistance that have emerged and selected in this aquatic environment have the potential of being transmitted by horizontal gene transfer to bacteria of the terrestrial environment, including human and animal pathogens (Sorum, 2006). Another problem created by the excessive use of antibiotics in industrial aquaculture is the presence of residual antibiotics in commercialised products (Sorum, 2006). This problem has led to unintended consumption of antibiotics by consumers with the added potential alteration of their normal flora that increases their susceptibility to bacterial infections and also selects for antibiotic-resistant bacteria. Moreover, unintended consumption of antibiotics in food can generate problems of allergy and toxicity, which are difficult to diagnose because of a lack of previous information on antibiotic ingestion.

Pesticides were introduced after the World War II for their various benefits but general worldwide intensive usage now poses potential hazards to the environment and human health (Chambers et al., 2001). In Vietnam, the insecticide endosulfan, commonly known by its trade name thioldan, is still used in agriculture and aquaculture, despite its prohibition (Tu et al., 2006). Joshi and Mukhopadhyay (1980) showed that the 48 h LC50 value of endosulfan (35% purity) in juvenile black tiger shrimp was 12.2 g/L. At low concentrations in water, sublethal effects such as altered energy metabolism (Mishra and Shukla, 1995) and ion toxicity conclusions (Singh et al., 2002) have been reported. The long-term ecological hazards associated with the use of organochloride, organophosphate and carbamate pesticides propelled the introduction of a new generation of pesticides with a lesser degree of persistence. In this direction, the use of pyrethroids as insecticidal and antiparasitic formulations has markedly increased as a viable substitute and account for over 30% of insecticides used globally (Prasanthi et al., 2005). Among pyrethroid, deltamethrin has a potent insecticidal activity with an appreciable safety margin (Mestres and Mestres, 1992). It kills insects by contact and works by paralysing their nervous system and therefore gives a quick knock down effect (Velisek et al., 2007). The rapid disappearance of deltamethrin from the water and its low bioconcentration capacities indicate that this molecule will not accumulate through the food chain. Nevertheless, its high toxicity and rapidity of action could cause significant harm to aquatic animals after direct treatment. L’Hotellier and Vincent (1986) showed that the 96h LC50 value of deltamethrin (25% purity) in pink shrimp (*Penaeus duorarum*) was 0.35 g/L. However, there is no data concerning its acute toxicity in black tiger shrimp, even if Smith and Straton (1986) showed that lobster and shrimp are susceptible to all pyrethroids. In Mekong River Delta, Vietnam, deltamethrin is extensively used in black tiger shrimp farms for treatment of water quality and reduction of disease problems (Tu et al., 2006), thus creating the possibility that this pesticide might still be found in the environment. The widespread use of these pesticides in Vietnamese shrimp farms consequently leads to the exposure of manufacturing workers, field applicators and the surrounding ecosystem, potentially impairing the health of shrimp and, finally, humans who eat those products.

In Vietnam, pesticide concentrations in the environment are not documented in the yearly environmental reports and information on this type of contaminations is generally lacking. The impacts of pesticide contaminations on aquatic ecosystems have been well studied in North America, Japan and many parts of Europe. In contrast, there is very little data on the levels of pesticide residues in developing countries.

Comprehension of the mechanisms related to the sublethal effects caused by these chemicals upon shrimp metabolism would help to develop sensitive and precise diagnostic tools with a predictive capability in assessing the toxic effects, thus contributing to better pond management (Bainy, 2000). The inhibition of acetylcholinesterase (AChE) is a useful biomarker of organophosphate and carbamate pesticides (Fulton and Key, 2001). However, several studies have indicated that AChE is also sensitive to metals, detergents and complex mixtures of pollutants (Gill et al., 1990; Payne et al., 1996; Guilhermino et al., 1998, 2000). Cholinesterase activity (ChE) are typically subdivided into two isoforms: acetylcholinesterase and butyrylcholinesterase (BChe) or pseudocholinesterase (Massoulie and Tantout, 1998). These two isoforms can be distinguished by their substrate preference and behaviour towards selective inhibitors (Massoulie et al., 1993). AChE is known to play a major role in cholinergic neurotransmission, hydrolysing the neurotransmitter acetylcholine at cholinergic synapses (Talesa et al., 1992), but the role of BChe remains to be clarified (Kozlovskaya et al., 1993). Since the properties of ChE may differ between species, it is important to characterise the type of enzyme present in the species studied before using it as a biomarker (Kristoff et al., 2006). Considering the above arguments, the aims of the present study were (i) to characterise the ChE activity present in muscle and gills of black tiger shrimp using different substrates and selective inhibitors and (ii) to assess the potentiality to use AChE activity in shrimp as a biomarker of exposure to antibiotics and pesticides commonly used in Vietnamese aquaculture.

### 2. Materials and methods

#### 2.1. Chemicals

Antibiotics (enrofloxacin, furazolidone; 98% purity), pesticides (endosulfan, deltamethrin; 98% purity), potassium phosphate dibasic (K2HPO4), potassium phosphate monobasic (KH2PO4), sodium phosphate dibasic (Na2HPO4), sodium phosphate monobasic (NaH2PO4), bovine serum albumin (BSA), acetylthiocholine iodide (AChI), propionylthiocholine iodide (PThI), S-butyrylthiocholine iodide (BThI), 5,5’-dithio-2-bis-nitrobenzoate (DTNB), eserine sulphate, tetra-(mono-isopropyl) pyrophosphor-tetra-mide (iso-OMPA), 1,5-bis(4-

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Plakas et al., 1994) and in Nile tilapia (*Oreochromis niloticus*) (Xu et al., 2006). In Vietnam, these antibiotics are used during the culture of shrimp, mainly to prevent (prophylactic use) and treat (therapeutic use) bacterial diseases. Enrofloxacin and furazolidone are generally blended with feed at dose of 4–5 g/kg feed for 7 days (Tu et al., 2006). The usage of antibiotics has brought about great concern. The unconsumed food and shrimp faeces containing antibiotics reach the sediment or can be washed by currents to distant sites. Once in the environment, these antibiotics can be ingested by wild fish, shrimp and other organisms. These residual antibiotics can remain in the sediment, exerting selective pressure, thereby altering the composition of its microflora and selecting for antibiotic-resistant bacteria. The determinants of antibiotic resistance that have emerged and selected in this aquatic environment have the potential of being transmitted by horizontal gene transfer to bacteria of the terrestrial environment, including human and animal pathogens (Sorum, 2006). Another problem created by the excessive use of antibiotics in industrial aquaculture is the presence of residual antibiotics in commercialised products (Sorum, 2006). This problem has led to unintended consumption of antibiotics by consumers with the added potential alteration of their normal flora that increases their susceptibility to bacterial infections and also selects for antibiotic-resistant bacteria. Moreover, unintended consumption of antibiotics in food can generate problems of allergy and toxicity, which are difficult to diagnose because of a lack of previous information on antibiotic ingestion.

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2.3. Enzyme assays

The ChE activity was determined in triplicate at 25°C, according to the colorimetric method initially developed by Ellman et al. (1961). The reaction mixture was prepared in 50 mM Na₂HPO₄/NaH₂PO₄ at pH 7.4 containing ACh and DTNB at a final concentration of 1.5 and 150 mM, respectively. In total, 50 µL of PMF was added to start the reaction. AChE activity was recorded using a spectrophotometer (Model BioMate 5, Thermo Electro, UK) at 412 nm for 10 min. AChE activity was expressed in nanomoles of hydrolysed acetylcholine iodide/mg protein. Protein concentration was determined following the method of Lowry et al. (1951).

2.4. Effects of antibiotics and pesticides on AChE activity

Animals: Shrimp (average weight of 8.8–10.0 g) were obtained from extensive shrimp farms in Camau province, Vietnam. Upon arrival, shrimp were acclimated to laboratory conditions in composite tanks (capacity of 2 m³) filled with natural aerated water of 15 g/L and 30–32°C for 2 weeks. Shrimp were fed with commercial shrimp feed (35% crude protein), four times daily, but were starved 1 day before experiment.

2.4.1. Antibiotic medication

Enrofloxacin or furazolidone powder was mixed with commercial shrimp feed at 4 g/kg. Medication was coated with 3% water and then with 3% squid oil and kept frozen at -20°C until use. Seventy shrimp per tank were stocked in nine composite tanks (capacity 2 m³) filled with natural aerated water of 15 g/L and 30–32°C for 2 weeks. Shrimp were fed with commercial shrimp feed (35% crude protein), four times daily, but were starved 1 day before experiment. Shrimp were sampled before medication (day 0), after 7 days medication and after 7 days post-medication.

2.4.1.1. Determination of antibiotics in shrimp muscle

Muscle samples were analysed for enrofloxacin and the furazolidone metabolite, 3-amino-2-oxazolidinone (AOZ), by liquid chromatography mass spectrometry (LC–MS). A liquid–liquid extraction methodology with acetonitrile for enrofloxacin and ethyl acetate for AOZ was used. Separation and detection of enrofloxacin and AOZ were done with a 2690 Waters high-performance liquid chromatography and a Micromass Triple Quadrupole mass spectrometer (Micromass, Manchester, UK). Samples were analysed on an electrospray source. The detection limit for enrofloxacin and AOZ was 10 and 0.1 µg/kg, respectively. The method has been validated according to Commission Decision 2002/657/EC and ISO 17025 requirements.

2.4.2. Pesticides exposure

Endosulfan or deltamethrin was dissolved in acetone to prepare a stock solution of 0.1 mg/mL. Twenty-five shrimp per tank were stocked in twenty-four composite tanks (capacity 500 L) filled with 200L of 15 g/L natural water. Six tanks served as control treatments. Nine other tanks were exposed to three sublethal concentrations of endosulfan (0.01, 0.1 or 1 g/L), while the last nine tanks were exposed to three sublethal concentrations of deltamethrin (0.001, 0.01 or 0.1 µg/L) for a period of 4 days. Afterwards shrimp were brought back to chemical-free water during 7 days for recovery. During the 4 days exposure, water in each tank was daily siphoned out and replaced by freshly prepared water with the same concentrations of pesticide. Shrimp were starved during exposure, but fed with commercial shrimp feed during recovery. All treatments and controls received the same aceton concentration (0.01%). This aceton concentration is below the no-observed-effect concentration (NOEC) of 0.1% reported by Mayer (1987). Water samples were collected everyday, at 3 and 24 h after the water renewal for analysis of nitrates and nitrites. No mortality was observed during the experiment. Shrimp were sampled before exposure (day 0), after 4 days exposure and after 7 days post-exposure.

2.4.2.1. Determination of endosulfan and deltamethrin in water

The chemicals were extracted from water samples by solid phase extraction according to the method described by de la Colina et al. (1996) using a Supelco Supelclean™ ENV18 SPE Tubes 6 mL (1 g) (Supelco, Bellefonte, PA, USA). Endosulfan and deltamethrin retained on the column were eluted with 5 mL of an isooctane/ethyl acetate solution (85:15:v/v). For water samples, the final elution was evaporated under a gentle stream of nitrogen to a volume of 50 µL using a Supelco Visteon™. The purified extracts were analysed by high-resolution gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a N65 ECD detector (Thermo Quest, Milan, Italy) and an autosampler for liquid samples Thermo Quest AS 2000 (Thermo Quest, Milan, Italy). Two microtitre of the solution were injected by means of a cold “on-column” injector. The chemicals were separated on a 30 m × 0.25 mm (0.25 µm film thickness) Restek RxI-5ms capillary column (Restek, Bellefonte, PA, USA). Identification and quantification of pesticides were performed using the Chromad 2.2 (Fisons Instruments, Thermo Quest, Milan, Italy) software for Windows. The limit of detection of endosulfan and deltamethrin was 0.5 pg/L. The limit of quantification of endosulfan and deltamethrin was 1.7 pg/mL (ppt).

2.4.2.2. Actual concentrations of endosulfan and deltamethrin in water

Endosulfan and deltamethrin concentration in water were analysed using the method described above. The actual concentrations were 0.009, 0.09 or 0.9 µg/L for endosulfan and 0.0007, 0.007 or 0.07 µg/L for deltamethrin.

2.5. Sampling materials and biochemical analysis

At each sampling time, muscle and gills from 6 shrimp per tank were collected on ice, pooled per tank and kept at –80°C until AChE activity measurement (the method described in Section 2.3).

2.6. Statistical analysis

All results were expressed as mean ± standard deviation (SD). Data were first checked for normality (Shapiro–Wilks’s test) and homogeneity of variance (Levene test). All tests were performed using the software Statistica 5.5 (Statsoft, Inc., 2000).

To determine the kinetic parameters, i.e., the apparent Michaelis–Menten constant (Kₘ) and the maximum substrate hydrolysis velocity (Vₘₐₓ), substrate concentration versus reaction velocity curves were analysed using the Solver feature of Microsoft Excel by fitting experimental data to the Michaelis–Menten equation (http://www.biol.paisley.ac.uk/kinetics). The no-observed-effect concentration and the lowest-observed-effect concentration (LOEC) were calculated for each inhibitor using a one way analysis of variance (ANOVA), followed by Dunnette’s test. The significance level was 5%.

For antibiotic and pesticide studies, data were analysed using repeated measures analysis of variance (two-way ANOVA) following the general model summarised by Paine (1996). This model was chosen because of the repetitive sampling within the same tanks, meaning the non-independence of the groups. AChE activity was assessed as a result of treatments (control, enrofloxacin and furazolidone) or actual sublethal concentrations (0; 0.009; 0.09 and 0.9 µg/L endosulfan or 0; 0.0007; 0.007 and 0.07 µg/L deltamethrin), replicate tanks (within treatments), sampling times within replicates (day 0; 7 days medication; 7 days post-medication for antibiotics and day 0; 4 days exposure and 7 days post-exposure for pesticides). If a time–treatment interaction was significant, a different time effect was observed depending upon the treatment, meaning that antibiotic or pesticide affects AChE activity during the exposure period. In case a significant interaction was detected with the ANOVA test, a multiple comparison test (Fisher LSD test) at a 5% significance level was used to examine differences between groups.

3. Results

3.1. ChE characterisation

AChE activity in both muscle and gills of shrimp showed preference for AChT to other substrates (AChT > BUTH, PRTH), with higher activities in muscle than in gills at all assayed concentrations (Fig. 1). The highest activity of 3223 ± 79 nmol/min/mg protein (muscle) and 571 ± 29 nmol/min/mg protein (gills) was obtained with 1.5 mM AChT substrate. Relative to AChT, the percentage of activity in muscle and gills was about 62% for BUTH and 61–65% for PRTH. Michaelis Kₘ values present the substrate concentrations (µM) required to obtain half of Vₘₐₓ (nmol/min/mg protein). The estimated ChE kinetic parameters for each substrate were reported in Table 1. These results support the hypothesis that the enzyme has a marked preference for AChT. Based on these findings, to ensure that the concentration was not rate limiting over a 10 min reaction period, we defined 1.5 mM of AChT substrate as the optimal concentration for AChE activity measurement.

Fig. 2 illustrates the effects of three inhibitors on ChE activity using AChT as substrate. Exsine sulphate showed almost full inhibition on ChE activity for both tissues (LOEC_ and gills = 0.005 mM).
BW284c51 also caused an 80% and 90% ChE inhibition in muscle and gills, respectively, as the concentration increased to 2 mM (LOEC\textsubscript{muscle} = 0.05 mM, LOEC\textsubscript{gills} = 0.005 mM). No significant effects were observed with iso-OMPA in both tissues (p > 0.05). In all experiments with selective inhibitors, no significant differences between ultra pure water and ethanol controls were found.

### 3.2. Effects of antibiotics on AChE activity

During the experiment, salinity and temperature ranged between 15.2 and 15.6 g/L and between 29.8 and 31.5 °C. We observed that AChE activity was not significantly affected in muscle (Fig. 3b) for shrimp fed with enrofloxacin (treatment–time interaction, $p = 0.76$) or furazolidone (treatment–time interaction, $p = 0.31$). On the other hand, AChE activity significantly decreased (up to 28%) in gills of shrimp fed with furazolidone (treatment–time interaction, $p = 0.008$) (Fig. 3a), while it was not significantly changed over time for controls and shrimp medicated with enrofloxacin (treatment–time interaction,
ments at any sampling time (these parameters were not significantly different between treatments).

Moreover, we could not detect any effect in muscle of shrimp exposed to endosulfan or deltamethrin (Figs. 4a and 5a). Significant differences in each treatment taken independently (p < 0.05). If a significant time–treatment interaction effect was observed, different letters mean significant differences in each treatment taken independently (p < 0.05).

3.3. Effects of pesticides on AChE activity

During the experiment, salinity and temperature ranged between 14.8 and 15.1 g/L and between 28.5 and 31.0 °C. At 3 h after water renewal, the concentrations of nitrates and nitrites (mean ± SD) were 1.84 ± 0.23 and 0.02 ± 0.01 mg/L for control treatment, 1.92 ± 0.22 and 0.02 ± 0.01 mg/L for endosulfan treatment and 1.98 ± 0.25 and 0.02 ± 0.01 mg/L for deltamethrin treatment. At 24 h after water renewal, the concentrations of nitrates and nitrites (mean ± SD) were 1.98 ± 0.15 and 0.02 ± 0.01 mg/L for control treatment, 2.11 ± 0.25 and 0.03 ± 0.01 mg/L for endosulfan treatment and 2.01 ± 0.30 and 0.03 ± 0.01 mg/L for deltamethrin treatment. The mean values of these parameters were not significantly different between treatments at any sampling time (p > 0.05).

No significant change of AChE activity was observed in gills of shrimp exposed to endosulfan or deltamethrin (Figs. 4a and 5a). Moreover, we could not detect any effect in muscle of shrimp exposed up to 0.09 µg/L endosulfan (treatment–time interaction, p = 0.89) (Fig. 4b) or up to 0.007 µg/L deltamethrin (treatment–time interaction, p = 0.95) (Fig. 5b). However, a significant inhibition compared with the control was observed in muscle after 4 days exposure to 0.9 µg/L endosulfan (30% inhibition) or 0.07 µg/L deltamethrin (49% inhibition) (treatment–time interaction, p = 0.006 for endosulfan treatment and p = 0.02 for deltamethrin treatment). No recovery was observed after 7 days post-exposure to 0.9 µg/L endosulfan (Fig. 4b), while total recovery occurred after 7 days post-exposure to 0.07 µg/L deltamethrin (Fig. 5b).

4. Discussion

ChE activity of shrimp showed in both muscle and gills preference for ACTH compared with other substrates. Similarly to our results, the substrate ACTH was preferred by ChE for estuarine crab (Chasmagnathus granulatus), grass shrimp (Palaeomonetes pugio), white shrimp (L. vannamei), and common prawn (Palaeamon serratus) (Monserrat and Bianchini, 1998; Key and Fulton, 2002; Frasco et al., 2006; García-de la Parra et al., 2006). Although most of the studied ChE from invertebrates display low activity towards BUTH, it was the most suitable substrate for lobster (Pilumnus vulgaris) ChE (Talesa et al., 1992). In another study, the substrate PRTH was preferred by ChE for brine shrimp.
et al., 1990), grass shrimp (Key and Fulton, 2002) and whiteleg shrimp (García-de la Parra et al., 2006). These results are in agreement with findings that have been reported for other shrimp, such as common prawn (Bocquene et al., 1992). In addition, for a particular species, the preferred substrate can vary depending on tissues. For example, Frasco et al. (1988) obtained with PRTH and BUTH substrates were about 54% and 6%, respectively, of the activity observed with ACTH (Habig et al., 1988). In contrast, the most suitable substrate for body crab (Maria verrucosa) was PRTH followed by ACTH and BUTH (Talesa et al., 1992). In addition, for a particular species, the preferred substrate can vary depending on tissues. For example, Frasco et al. (2006) found that the preferred substrate of ChE present in common prawn eyes was ACTH, whereas Bocquene et al. (1990) found that PRTH was the most suitable substrate in a whole extract of this species.

In the present study, enzymatic activity measured in both muscle and gills was almost inhibited by eserine sulphate, suggesting that the enzyme assayed in our experimental conditions was mainly a ChE. Based on these results and the fact that both muscle and gills were sensitive to BW284c51, but not to iso-OMPA, we can conclude that ChE in both tissues actually was AChE. These results are in agreement with findings that have been reported for other shrimp, such as common prawn (Bocquene et al., 1990), grass shrimp (Key and Fulton, 2002) and whiteleg shrimp (García-de la Parra et al., 2006).

Currently, there are no reports on the toxicity of enrofloxacin and furazolidone on AChE activity in shrimp. Contrarily to the cited studies above, we exposed the shrimp under realistic conditions, by mixing the antibiotic with feed at a ratio that is widely used by Vietnamese farmers. Enrofloxacin seems to have no impact on AChE activity, on the analysed organs. At the opposite, up to 28% decrease was observed in gills for shrimp fed with furazolidone, even after 7 days post-medication. Several investigations have demonstrated that the use of furazolidone caused deleterious effects on animal health. This nitrofuran has been shown to induce hypotension, to produce disordered hepatic metabolism, anorexia and growth reduction in turkey (Staley et al., 1978; Simpson et al., 1979), and to decrease glutathione and ascorbic acid levels, while increasing lipid peroxidation level in rats (Ali, 1992). Compared with enrofloxacin, furazolidone showed lower elimination rate, which might explain that it had more significant effects. The results from enrofloxacin and AOZ residues showed that the maximum concentration for AOZ and enrofloxacin in muscle were 874 ± 326 and 441 ± 274 µg/kg after 7 days medication, respectively. This level decreased quickly after the last feeding with enrofloxacin, and dropped to 17 ± 6 µg/kg at 7 days post-medication, while it was still at 305 ± 120 µg/kg after 7 days post-medication for furazolidone. The reason why furazolidone affects AChE activity in gills, but not in muscle, is unknown. However, furazolidone mixed with food might be leaching into the water, which could cause direct effects in gills. Moreover, Sancho et al. (1997) pointed out that gills are the first organ to be exposed to waterborne contaminants and the primary site for xenobiotics absorption due to its large surface area and permeability. Thus, gills can be considered as a good candidate to early assess the effects of antibiotics.

Nitrofurans are synthetic antibiotics, characterised by their basic chemical structure (nitrofuran ring) that are frequently used as veterinary drugs because of their broad antibacterial spectrum. They have been banned for food producing animals by the European Union and the US Food and Drug Administration due to their carcinogenic and mutagenic properties. Nevertheless, furazolidone has still been found in imported poultry and prawns. Because nitrofurans are light sensitive and undergo rapid metabolisation in animals, parent nitrofurans are not detected in most food products. The methods for detection of nitrofurans must be based on the detection of tissue-bound metabolised residues. The side chains of bound metabolites, such as 3-amino-2-oxazolidone from furazolidone, are more stable than their parent drug, hence used as targeted molecules for detection.

Fluoroquinolones are a subset of broad-based antibiotics belonging to the quinolone family. They have been used successfully to treat salmonellosis and have also been proving their usefulness for treatment of infections caused by multiple antibiotic-resistant strains. Side effects became more evident in the 1980s and use of fluoroquinolones has become an important public concern. Quinolone-resistant Salmonella can also be cross-resistant to other agents including chloramphenicol and tetacycline.

We showed that AChE activity was inhibited in shrimp muscle by 30% after 4 days exposure to 0.9 µg/L of endosulfan. This inhibition still remained even 7 days post-exposure. Similarly, Saravana and Geraldine (2000) reported that intermittent juveniles of prawns (Macrobrachium malcolmsonii) exposed to 32.0 ng/L of endosulfan for a period of 21 days had muscle AChE activity inhibited by 27%. However, in juvenile bluegill sunfish (Lepomis macrochirus) exposed to 1 µg/L endosulfan for 96 h, AChE activity was inhibited by 16.31% (Dutta and Arends, 2003). This is in accordance with the conclusion of Couch (1978), who found that penaeid shrimp were more sensitive to most pesticides than fishes and molluscs. On the other hand, in shrimp exposed to

![Fig. 5. AChE activity in gills (a), and muscle (b) of shrimp following 4 days exposure and 7 days post-exposure to deltamethrin. Values are mean ± SD (n = 3). Significant effect at p < 0.05. If a significant time–treatment interaction effect was observed, different letters mean significant differences in each treatment taken independently (p < 0.05).](image-url)
0.07 µg/L of deltamethrin for 4 days, the AChE activity in muscle was inhibited by 49%, while carp (Cyprinus carpio) exposed to 2 µg/L of this pesticide for 3 days displayed non-significant AChE inhibition in skeletal muscle (Szegletes et al., 1995). The present study indicates that shrimp can recover 7 days after deltamethrin exposure. Deltamethrin has been classified “immobile” by the US EPA (URL 2), because of its strong adsorption on particles and its low solubility in water. The degradation of deltamethrin to less toxic products is rapid. Therefore, most of the affected organisms show rapid recovery. In general, the toxic mode of action for pyrethroids is the disruption of neuronal impulse conductance which results in the generation of multiple action potentials that leads to tremors and in coordination. These effects, observed at a cellular level, can lead to various health concerns (He, 1994).

On the contrary to deltamethrin exposure, no recovery from depressed AChE activity occurred for shrimp exposed to endosulfan. Yasmeen et al. (1991) explained that endosulfan is lipophilic and is easily diffused into cells where it can disrupt the normal functioning of cell proteins and inhibit the action of certain enzymes. Results from this study give the idea that 7 days post-exposure is not sufficient for the restoration of normal AChE activity. This may cause exposed shrimp to be more susceptible to other anthropogenic or natural hazards and likely affects their normal behaviour. Several studies noted that recovery of AChE inhibition occurred after exposure to phosphamidon and methylparathion in penaeid prawn (Metapenaeus monoceros) (Reddy and Rao, 1988), to profenofos in Australian freshwater shrimp (Paratya australiensis) (Abdullah et al., 1994) and to dichlorvos in larvae of the lobster (Homarus gammarus) (McHenery et al., 1996). The difference between effects of endosulfan and deltamethrin on AChE activity must be taken into account when making diagnoses based on field samples. Samples from a suspected deltamethrin contamination should be analysed immediately.

There was no general agreement on the degree of AChE inhibition, which is likely to be associated with toxic manifestation and death. In the present study, no mortality was observed during 4 days exposure even if AChE activity in muscle was inhibited by more than 30% in shrimp exposed to endosulfan or deltamethrin. Coppage et al. (1975) reported that 70–80% inhibition of brain AChE was associated with mortality in most species. Chin and Sudderuddin (1979) observed that after more than 80% inhibition of brain AChE, common carp fingerlings still survived. However, reduction in the survival rate of salmonid fish was reported as a consequence of 50% inhibition of AChE (Post and Leasure, 1974).

Recently, there has been evidence that contaminants other than organophosphate and carbamate pesticides may inhibit the activity of the enzyme AChE both under in vitro and in vivo conditions. The results presented in the present study suggest that the use of AChE as a specific biomarker for organophosphate and carbamate pesticides should be questioned and that the use of this enzyme as a biomarker could be extended. In general, it seems that no individual biomarker can give a complete diagnosis of pollution (Lagadic et al., 2000). Rather, a set of biomarkers at different levels of biological organisation is required to be effectively applicable in a biomonitoring programme.

5. Conclusions

The present study highlighted the fact that AChE represents a future and cost effective biomarker to assess the health and the stress level of shrimp exposed to drugs and chemicals in Vietnamese ponds. Muscle AChE should be assessed to point out endosulfan or deltamethrin exposure, while gill AChE impairment could indicate furazolidone exposure. However, care should be taken while using AChE activity alone. First, pesticide effects were observed at the highest tested concentrations only, suggesting that AChE activity is not sensitive enough to detect low pesticide exposure. Second, a transient effect was noticed for deltamethrin exposure since 7 days post-exposure permitted a total recovery. This fact limits the use of AChE activity to detect exposure to this pyrethroid to shrimp sampled just after the exposure. In conclusion, AChE activity can indicate exposure to pesticides and antibiotics in black tiger shrimp and can reveal health impairment. However, AChE should be used in association with other biochemical, physiological and molecular biomarkers. This multiplex approach is the only method able to bring out accurately with sensitivity the past or present exposure of shrimp to drugs and chemicals used in tropical aquaculture.

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References


