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Notes

Effects of Phenanthrene Exposure on the Acetylcholinesterase Activity of Olive Flounder (*Paralichthys olivaceus*)

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Acetylcholinesterase (AChE) activity is a potential biomarker for phenanthrene exposure in aquatic organisms. Olive flounder (*Paralichthys olivaceus*) were exposed to three different concentrations (0.5, 1.0 and 2.0 μM) of phenanthrene for four weeks. AChE activities in the brain, heart and eyes were documented. Inhibition of AChE activity was found significant in flounder treated with a concentration greater than 1.0 μM of phenanthrene. This indicates that a chronic exposure to phenanthrene induces damage in various organs (brain, heart and eyes) and changes of AChE activities might be a useful biomarker to assess the impacts induced by polycyclic aromatic hydrocarbon (PAH). Evidence from this study confirms that the measurement of AChE in the brain and eyes of flounder is a valuable tool that along with other biomarkers can maximize an ecotoxicologists' confidence in assessing the impacts of oil and PAH pollution in the aquatic environment.

Key words: Acetylcholinesterase, Biomarker, *Paralichthys olivaceus*, Phenanthrene

Aquatic environments have been used for decades as a major repository of anthropogenic wastes. Among the types of pollutants typically attributable to human activities, petroleum products are one of the most ecotoxicologically relevant. Polycyclic aromatic hydrocarbons (PAHs) have served as markers of fish exposed to oil pollution (Ogata and Miyake, 1979) and some PAHs are known as carcinogens (Andelman and Snodgrass, 1974). One of the biomarkers most frequently used in fish for the diagnosis of exposure to organophosphate and carbamate compounds is the measurement of the inhibition of enzyme cholinesterase (Sancho et al., 2000). Although Acetylcholinesterase (AChE) inhibition has been widely monitored in terrestrial and coastal aquatic environments as a biomarker of aquatic toxicants, including organophosphorus (Holland et al., 1967), the effect of phenanthrene on AChE has not yet been clear. Acetylcholine is one of the most important neurotransmitters in either central or peripheral nervous systems and the inhibition of AChE has been proposed as biomarker for the neurotoxicity (Manzo et al., 1995). The AChE marker for aquatic pollution has gained a wide acceptance in environmental studies of both fresh and marine waters over the past few years. The activity of this enzyme was used to assess the toxicity of existing discharges into aquatic ecosystem (Martinez-Tabche et al., 1998). In this study, we tried to elucidate the effect of phenanthrene on AChE activity in brain, heart and eyes in olive flounder (*Paralichthys olivaceus*).

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Olive flounder (*P. olivaceus*), 51±4.3 g mean body weight, were obtained from a local fish farm in Korea, transferred to a tank and kept at 20±1℃. Flounder were acclimated to the laboratory condition for several weeks prior to experiment. Fish were maintained on a 12 h light/dark cycle at all times. Test chamber (glass aquaria, 120 L capacity) were filled with 80 L of sea water. Water characteristics, measured by the method described in APHA (1995), were as follows: pH, 8.03±0.4; temperature, 20±1℃; salinity, 31.8±0.7‰ and dissolved oxygen 7.5-7.8 mg/L. Phenanthrene was initially dissolved in ethanol (Sigma Chemical, St. Louis, MO) to obtain initial stock solution. Prior to introduction of fish to test aquaria, solutions of phenanthrene (>96% purity, Sigma Chemical, St. Louis, MO) working solution

were mixed with filtered seawater to attain a nominal concentration of 0.5, 1.0 and 2.0 μM. The working solutions were stirred 8-16 h prior to usage. In each test chamber, a group of 10 fish was exposed for a period of 2 and 4 weeks under semi-static conditions with aeration to maintain dissolved oxygen levels over 75% saturation. The phenanthrene dose was renewed every second day along with the seawater. AChE Activity was determined in (1:50) brain and heart homogenate in 0.1 M phosphate buffer containing 0.1% Triton X100 (Sigma), pH 8. Whole eyes homogenates (1:100) were also prepared in the same buffer. The crude homogenates were then decanted into Eppendorf tubes and centrifuged at 10,000×g for 20 min at 4℃. The supernatants were removed and used to test AChE activity. AChE activity was measured by an automated adaptation of the Ellman assay (Ellman et al., 1961) using a spectrophotometer (Hach 4000, USA). AChE activity was normalized to protein content and expressed as U min^{-1} mg $protein^{-1}$ (1U = 1 milli Optical Density (mOD) unit). Protein concentration was determined using Bradford's method (1976), with a bovine serum albumin (Sigma, USA) as standard. Statistical analyses were performed using SPSS/PC + statistical package. Significant differences between groups were determined using one-way ANOVAs and Duncan's test for multiple comparisons or the Student's t-test for two groups (Duncan, 1955). Significance level was established at $P<0.01$.

In this study, the inhibition degree of enzyme activity increased significantly ($P<0.01$) with the increase of phenanthrene level (>1.0 μM) and exposure time at 4 week (Fig. 1). Among tissues examined, brain is supposed to be a major part of body which is seriously damaged by phenanthrene exposure since the percentage inhibition of AChE was significantly higher than heart and eyes when exposed to 1.0 μM (40.05%) or 2.0 μM (66.60%) of phenanthrene for 4 weeks. AChE activity in eyes was affected significantly by phenanthrene but the activity in heart was not declined as much as in brain and eyes. Eyes seems to be the first affected organ as the highest reductions of AChE activity than any other organs were already observed at 2 weeks in 1.0 and 2.0 μM exposed groups (34.53% and 37.23%, respectively), and thereafter insignificant reduction was observed in 1.0 and 2.0 μM groups. Therefore, brain and eyes seem to be useful organs to monitor AChE activity following phenanthrene exposure. The high activity of AChE in brain lead to consider those organs useful for monitoring purpose, as is the case in other species (Bocquen et al., 1990). It has been hypothesized that suppression of AChE activity could be used as an indicator of stress of aquatic pollutants and their exposure (Bocquen et al., 1990; Kirby et al., 2000). Recently, Kang and Fang (1997) showed

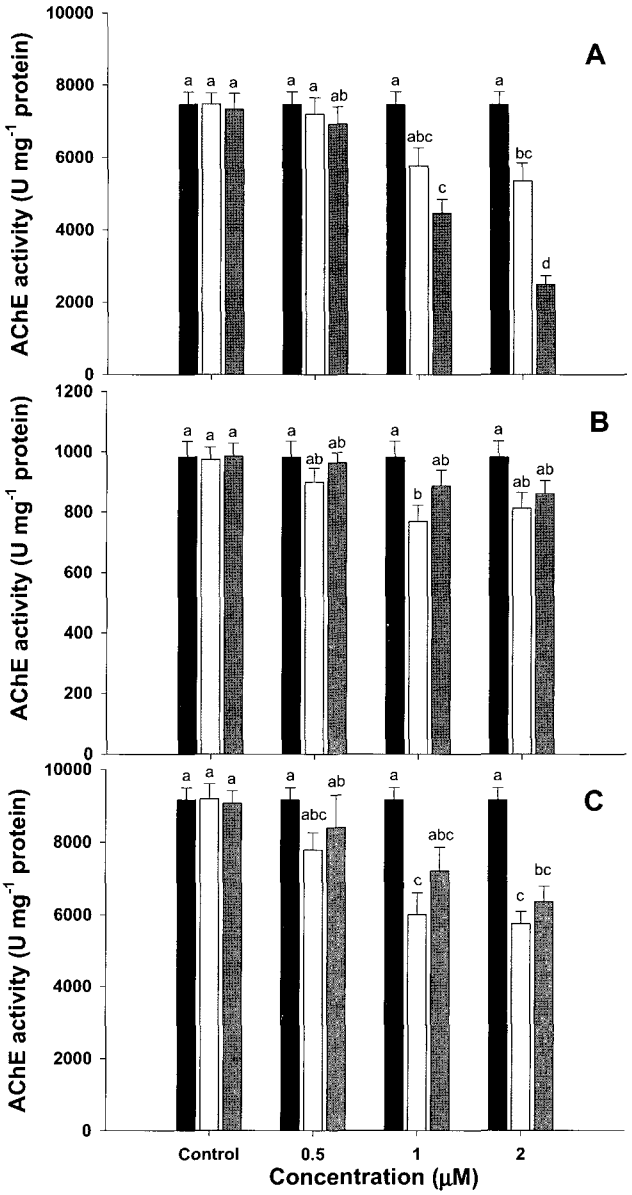

Fig. 1. Changes in acetylcholinesterase activity in brain (A), heart (B) and eyes (C) of *Paralichthys olivaceus* after phenanthrene exposure for 4 weeks. Values are means ± SE (n=10). Values with different superscript are significantly different ($P<0.01$) as determined as Duncan's multiple comparison.

that several PAHs directly inhibit AChE activity *in vitro*. Evidence from this study confirms that the measurement of AChE of brain and eyes of olive flounder is a valuable tool that should be incorporated into a series of biomarkers to maximize the confidence with which ecotoxicologists assess the impacts of oil and PAH pollution in the aquatic environment.

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References

Andelman, J.B. and J.E. Snodgrass. 1974. Incidence and significance of polynuclear aromatic hydrocarbons in the water environment. Crit. Rev. Environ. Control, 4(1), 69-83.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater, 19th edition. American Public Health Association, Washington, D.C., USA.

Bocquené, G., F. Galgani and P. Truquet. 1990. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. Mar. Environ. Res., 30, 75-89.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248-254.

Duncan, D.B. 1955. Multiple-range and multiple F tests. Biometrics., 11, 1-42.

Ellman, G.L., K.D. Courtney, V.J. Andreas and R.M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7, 88-95.

Holland, H.T., D.L. Coppage and P.A. Butler. 1967. Use of fish brain acetylcholinesterase to monitor pollution by organophosphorus pesticides. Bull. Environ. Contam. Toxicol., 2, 156-162.

Kang, J.J. and H.W. Fang. 1997. Polycyclic aromatic hydrocarbons inhibit the activity of acetylcholinesterase purified from electric eel. Biochem. Biophys. Res. Commun., 238, 367-369.

Kirby, M.F., S. Morris, M. Hurst, J.S. Kirby, P. Neall, T. Tylor and A. Fagg. 2000. The use of cholinesterase activity in flounder (*Platichthys flesus*) muscle tissue as a biomarker of neurotoxic contamination in UK estuaries. Mar. Pollut. Bull., 40(9), 780-791.

Manzo, L., A.F. Castoldi, T. Coccini. A.D. Rossi, P. Nicotera and L.G. Costa. 1995. Mechanisms of neurotoxicity: applications to human biomonitoring. Toxicol. Lett., 77(1-3), 63-72.

Martinez-Tabche, L., B.R. Mora, C. German-Faz and E. Diaz-Pardo. 1998. Acetylcholinesterase activity of the freshwater cladoceran, *Moina macrocopa*, used as an in vitro screening tool for water quality. Aquat. Ecosyst. Health Manage., 1, 317-322.

Ogata, M. and Y. Miyake. 1979. Disappearance of aromatic hydrocarbons and organic sulphur compounds from fish reared in crude oil suspensions. Water Res., 13, 75-78.

Sancho, E., J.J. Cerón and M.D. Ferrando. 2000. Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in *Anguilla anguilla*. Ecotoxicol. Environ. Saf., 46, 81-86.

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