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LDH/AChE and LDH/BChE Ratios (*Paralichthys olivaceus*) as Biomarkers of Coastal Pollution on Coast of Korea.

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This study was designed to develop biomarkers of coastal pollution using biochemical indices of flounder (*Paralichthys olivaceus*) by changes in lactate dehydrogenase (LDH) activity in the serum and cholinesterase activities in brain membranes. For this purpose acetylcholiesterase (AChE) activity, butyrylcholinesterase (BChE) activity, LDH/AChE ratio and LDH/BChE ratio of cultured flounders at 5 different sites on the southern coast of Korea were compared to those of wild flounders caught in the Pohang, eastern coast of Korea as a control group. Relatively high LDH activities were measured in the serum of flounders cultured on the southern coast of Korea (0.101~0.145 unit) than those in the Pohang control group (0.093 unit). AChE activities were significantly low (about 10~20%) in brain membranes of cultured flounders compared to those in the Pohang control group. The ratios of LDH/AChE and LDH/BChE were consistently higher (136~178%, 155~214%) in cultured flounders than those of Pohang control group. Thus, we propose that the ratios of LDH/AChE and LDH/BChE in flounders could be applicable for the diagnosis of marine pollution.

Key words: Flounder (*Paralichthys olivaceus*), Yellow Sea, Lactate dehydrogenase (LDH), Acetylcholinesterase (AChE), LDH/AChE, LDH/BChE, Butyrylcholinesterase (BChE) Biomarker

Introduction

Pollution in the marine environment has become an issue of grave concern to many coastal states. Marine environmental monitoring should provide information on the nature and extent of adverse impacts on the marine ecosystem and ocean's health. The measurement of contaminant loads alone cannot provide comprehensive quality criteria for the coastal environment. Biomarkers that can be used as a measure of health and fitness are essential when adverse effects of contaminants on the ecosystem are to be assessed.

Detection of low-level changes that underlie higher level effects and for which causality can be established, may provide early warning signs of impending environmental damage. Contaminant exposure in a number of bottom feeding marine fish species has been associated with a variety of adverse effects, including pathological conditions and physiological responses. Recently a number of promising biomarkers have been developed to assess contaminant exposure and the effects in indigenous fish (Stein et al., 1993). Various hematological parameters are reported to be very sensitive to intoxication by trace metals and organic microcontaminants (Everaarts et al., 1993).

A number of drugs and man-made toxic agents acts as irreversible enzyme inhibitors. Organophosphorus agents such as diisopropylphosphofluoridate (DIPF), malathion, and parathion bind to a specific serine residue within the active site of the enzyme acetylcholinesterase (AChE), thus inhibiting the enzyme (Maestro et al., 1980). The nonplasma specific enzymes such as lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) are present at considerably lower concentrations

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in plasma than in the tissues. However, the plasma concentration of these enzymes significantly increases during the various disease states (Korea Ocean Research and Development Institute, KORDI report, 1989). Although much research has been carried out in recent years to study the pollution-induced responses in fish, much effort still has to be done in order to incorporate these parameters in routine environmental monitoring programs.

We have already investigated and published in a bulletin the studies on the biochemical pollutant marker for diagnosis of marine pollution using flounder (*Paralichthys olivaceus*) in the Yellow and South Sea of Korea (Choi et al., 1997a-b; Moon et al., 1997; Choi et al., 1999a), on the biochemical pollutant index for diagnosis of marine pollution using flounder (*Pleuronichthys cornutus*) in the Yellow Sea and mussel (*Mytilus coruscus*) in the South Sea of Korea (Choi et al., 1997c-e; Choi et al., 1999b-c), and on the biochemical pollutant marker for diagnosis of marine pollution flounder (*Paralichthys olivaceus*) in the South Sea of Korea (Choi et al., 1998a-b). We also investigated the studies on the LDH/AChE and MDA/SOD ratios of flounder (*Paralichthys olivaceus*) as biomarkers of coastal pollution, and LDL-Chol/Hb and MDA/GSHPx ratios in the serum of flounder (*Pleuronichthys cornutus*) as biomarkers of marine pollution in the western coast of Korean (Choi et al., 1998a-b).

The purpose of this study is to test and validate biomarkers which can be used as powerful tools when assessing the marine environment. Sampling sites on the southern coast of Korea are affected by various pollutants input including trace metals and organophosphorus pesticides was chosen as sampling sites of experimental groups (Korea Ocean Research and Development Institute, 1989, 1997). Flounder is an abundant fish species in estuary and coastal areas and has been found to be satisfactory for biological monitoring along the Korean coast (Choi et al., 1997). This technique is based on the evaluation of the changes in the ratios of LDH/AChE and LDH/BChE of the flounder (*Paralichthys olivaceus*) caught on the southern coast of Korea. Thus, we analyzed lactate dehydrogenase (LDH) activities in the serum of flounders and cholinesterase (AChE and BChE) activities in brain membrane of flounders.

Materials and Methods

1. Sample Collection

Cultured flounders were collected in Gosung, Samchonpo, Hansando, Tonyong and Masan located on the southern coast of Korea. Wild flounders caught near Pohang on the eastern coast of Korea were chosen as a control group. These flounders were caught by the local fisherman in the area where habitat damage was the least probable. Seven flounder (*Paralichthys olivaceus*) samples were collected at each of the six sites ; wild flounder in Pohang (control) and cultured flounder (body length: 22.5~31.0 cm; body weight: 350~550 g).

2. Preparation of Serum and Brain Membranes

Flounders were anesthetized with *p*-aminobenzoic acid dissolved in ethanol. Blood samples were collected using Greenject-5 disposable syringe. The brain tissue samples were immediately excised,

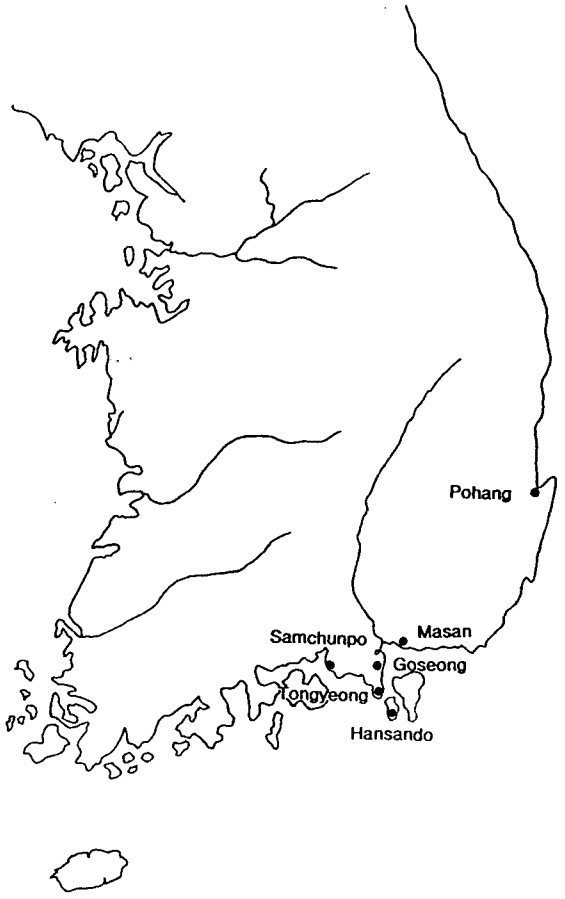

Fig. 1. Sampling stations of the flounder (*Paralichthys olivaceus*) used.

homogenized in phosphate buffer (1.15% KCl/10 mM phosphate buffer+5 mM EDTA, pH 7.4) and stored at -70℃. Brain membrane were prepared with 0.1 M TRIS buffer (pH 8.0) by previous method (Choi et al., 1998a). Protein analysis followed the method of Lowry et al. (1951).

3. Determination of LDH Activity

Lactate dehydrogenase (LDH) activities were determined by the method of Zimmerman et al. (1979) using kit reagents (Sigma Co., USA). LDH activity are based on the oxidation of lactate to pyruvate. 2.5 mℓ of LDH A (0.194 mM NADH, phosphate buffer, pH 7.5), 0.1 mℓ of LDH B (16.2 mM pyruvate) were added to 100 μℓ of serum. Absorbance of this mixture was read at 340nm during 3 minutes.

4. Determination of AChE and BChE Activity

Acethylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were determined by the method of Galgani and Bocquene (1991). 300 μℓ of 0.1 M Tris buffer (Trizma HCl+Trizma base, pH 8.0), 20 μℓ of 0.01M dithionitrobenzoic acid (DTNB), 10 μℓ of 0.1 M acetylthiocholine chloride were added to 10 μℓ of enzyme suspension in microplate well. This mixture read at 405 nm in a microplate reader (ELISA reader) for 5 minutes.

5. Data Analysis

Statistical analysis was done using the statistical package Super ANOVA. A onefactor First ANOVA was used to determine the overall significance of the data.

Results and Discussions

As shown in Table 1, LDH activities in the serum of flounders cultured on the southern coast ranged between 0.101~0.145 unit which were higher than those of the control group in Pohang (0.093 unit) ($p<0.01$~$p<0.001$). Relatively high LDH activity was found in flounders of Masan (0.145 unit) and Hansando (0.141 unit).

Serum LDH activity significantly increases during the various disease states in liver, heart and skeletal muscle etc (Choi et al., 1998c). If the health of the marine organisms are affected by various sources of pollutants discharged in the coastal environment, LDH activity might be one of the indicators responding to pollutants (Scheve, 1984).

AChE activities in the brain membrane of flounders ranged from 6,244 to 6,743 unit/min/mgP in cultured flounders. The above values were significantly lower than those of the control group in Pohang (7,936 unit/min/mgP) ($p<0.05$~$p<0.001$). The lowest AChE activity in the brain membrane of flounders was found in cultured flounders of Samchonpo (6,244 unit/min/mgP).

BChE activities ranged from 160 to 185 unit/min /mgP in the brain membrane of cultured flounders. Compared to the control group in Pohang (246 unit/min/mgP), BChE activities were significantly lower in cultured flounders ($p<0.05$~$p<0.001$).

AChE and BChE activities have been used as a useful indicator of contamination caused by organophosphorus pesticides (Choi et al., 1999a). Measurements along a pollution gradient in the North Sea have revealed great variations in AChE and BChE activity levels in the dab (*Limanda limanda*) (Choi et al., 1999b). As seen in Table 1, diagnosis of marine pollution may not be easy when considering only site-to-site variations of each individual biomarker.

Table 1 also shows the ratio of LDH activity in serum to AChE activity in the brain membrane

Table 1. LDH, AChE and BChE activities in flounders (*Paralichthys olivaceus*) of the southern coast of Korea.

Stations (Area)	LDH activity (unit/mℓ serum)	AChE activity (unit/mg pro.)	BChE activity (unit/mg pro.)	LDH/AChE ($\times 10^5$)	LDH/BChE ($\times 10^5$)
Control: East Sea					
Pohang (W)	0.093 ± 0.030	7,936 ± 472	246 ± 40	1.17 (100%)	40.0 (100%)
Experiments: Southen Sea					
Goseong (C)	0.110 ± 0.030[a]	6,743 ± 393[b]	162 ± 23[b]	1.63[b] (139%)	70.0[c] (175%)
Samchunpo (C)	0.109 ± 0.030[a]	6,244 ± 326[c]	160 ± 30[b]	1.75[c] (150%)	70.0[c] (175%)
Hansando (C)	0.141 ± 0.020[b]	7,245 ± 310[a]	185 ± 20[a]	1.95[c] (167%)	80.0[c] (200%)
Tongyeong (C)	0.101 ± 0.030	6,348 ± 308[c]	163 ± 20[b]	1.59[b] (136%)	62.0[c] (155%)
Masan (C)	0.145 ± 0.020[b]	6,973 ± 321[b]	169 ± 25[b]	2.08[c] (178%)	85.8[c] (214%)

The values are the mean ± SD with seven flounders per group. [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$ compared with control group.

of flounders. The ratios of LDH/AChE in cultured flounders were consistently higher (136~178%) than those of the control group in Pohang ($p<0.01 \sim p<0.001$). Highest LDH/AChE ratio was found in cultured flounders of Masan (178%). The ratios of LDH/BChE were also consistently higher in cultured flounders (155~214%) than those of the control group in Pohang ($p<0.01 \sim p<0.001$). Highest LDH/BChE ratio was also found in flounders of Masan (214%). Among the southern coastal areas both ratios were the lowest in Tongyong where extensive aquacultural activities are conducted under favorable conditions.

The ratios of LDH/AChE and LDH/BChE seemed to be more effective biochemical indicators of distress than AChE or BChE alone. Thus the ratio of LDH/AChE in flounders could be applicable for diagnosis of marine pollution.

Conclusion

Nowadays, there exists many types of biomarkers that can be used to detect the impacts of contaminants on marine biota. The biochemical analytic technique has been proven to be an efficient and a sensitive method for detection of contaminants for the above purpose. Adverse effects of pollutants on marine biota can be more conveniently detected when applying two indicators responding oppositely to the presence of pollutants.

Use of LDH/AChE ratio could be more reliable and sensitive since these indicators respond positively and negatively to environmental stress. In the same way LDH/BChE ratio could be a reliable indicator. Thus these ratios are proposed to be used as a biomarker for diagnosis of marine pollution. However, assuming that a biomarker has been sufficiently validatd to be included in monitoring programmes, the question remains of how to use the informations from biomarker research. Thus more frequent biochemical analysis; together with bioavailability studies of contaminants are needed to routinely monitor and assess the marine environment of the southern coast by sensitive biomarkers developed in this study.

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