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Regulation of Acetylcholine Esterase and Neurotransmitters in *Oryzias latipes* by Diazinon

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다이아지는 처리에 의한 송사리의 아세틸콜린에스터라제 활성 및 신경전달물질 함량의 변화

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ABSTRACT

Diazinon, an organophosphate pesticide, is relatively highly toxic to fish and causes vertebral malformation and behavioral change of fish at relatively low concentrations. To elucidate biochemical mechanism of the behavioral change of *Oryzias latipes* (killifish) caused by diazinon, the effect of the insecticide on acetylcholine esterase activities and the levels of some neurotransmitters were evaluated. Acetylcholine esterase activities in both head and body were significantly lowered at the concentration of 10 ppb of diazinon and acetylcholine contents in head tended to be upregulated with increasing concentration of diazinon. Exposure of killifish to 5000 ppb diazinon resulted in gradual decrease in acetylcholine content in body part with exposure time. Norepinephrine and serotonin concentrations in killifish head and body were highest at 1000 ppb of diazinon while neurotransmitter were relatively low in fish unexposed or exposed to lower dose of the pesticide, suggesting that increased norepinephrine and serotonin can partially account for diazinon-induced behavioral abnormality.

INTRODUCTION

Diazinon [O, O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate], is an organophosphorous insecticide widely used for the control of agricultural and household pests, the toxic effects of which are mainly due to the inhibition of cholinesterase.[1)] Although diazinon undergoes fairly rapid degradation in the environment, it may cause toxic effect on fish due to prolonged exposure with repeated input into the aquatic environment. This insecticide has a highly acute toxicity and can result in numerous poisonings in nontarget species. Diazi-

non is relatively highly toxic to fish, and it is well known that it causes vertebral malformation and behavioral change of fish at relatively low concentrations.[2),3)]

Behavioral change caused by pesticides is most likely related to changed levels in neurotransmitters such as acetylcholine, dopamine, serotonin, and norepinephrine.

Using killifish as model system the effect of diazinon on the levels of some neurotranmitters were investigated in this study.

MATERIALS AND METHODS

1. Experimental animals

Killifish (*Oryzias latipes*) was obtained from Toxicology Research Center, Korea Research Institute of Chemical Technology (Taejeon, Korea). One-year old fish were fed a commercially prepared flake diet (TetraMin, USA) once daily. Fish were held at 22± 2°C and 12L/12D light cycle to minimize reported seasonal variation in xenobiotic-metabolizing enzymes. Fish were held in a square glass chamber (45 × 24 × 30 cm) containing 30-liter of dechlorinated water (pH = 6.5 ~ 7.3) with aeration.

2. Chemical exposure

Diazinon (purity: 99%) was obtained from Wako pure chemical industry, LTD (Osaka, Japan). Fish were exposed to diazinon dissolved in dimethylsulfoxide for various times in a static environment. A vehicle control was run for each replication.

3. Tissue sample

Fish were quickly anaesthetized by submersing in chilled water and dissected into head and body. Tissues were homogenized (approximately 20 mg of tissue per ml of phosphate buffer (pH 8.0, 0.1 M)) in a Polytron homogenizer.

4. Acetylcholine esterase (AChE) assay

AChE activities of head and body were assayed in 45 mM phosphate buffer, pH 8.0, using 0.56 mM acetylthiocholine as substrate, according to Ellman.[4)]

5. Determination of neurotransmitter contents

Acetylcholine, norepinephrine and serotonin were assayed according to the methods described by Augustinson[5)] and Jacobowitz.[6)]

6. Protein

For all tissues, protein was quantified according to Lowry[7)] using bovine serum albumin as the standard.

RESULTS

1. Acetylcholine esterase activity

Fish were exposed to Diazinon at the concentrations of 0 ~ 5000 ppb for 24 hrs, followed by AChE assay. As shown in Table 1, acetylcholine esterase activities in brain were significantly reduced at the concentration of 10 ppb, and was further decreased to negligible level at the concentration of 1000 ppb of the pesticide. None of fish treated with 1000 ppb of diazinon was dead while 50% fish survived 5000 ppb of the pesticide. When fish were exposed to 5000 ppb diazinon for different times, brain acetylcholine esterase activity decreased from 69.7±7.8 nmoles substrate hydrolyzed per min per mg protein at time = 0 to 56.6±2.2 at time = 30 min while body acetylcholine esterase activity was decreased from 113.4±8.0 to 72.7±9.8 during the same period. Less than 10% enzyme activity of control remained after treated

Table 1. Changes in acetylcholine esterase activity of *Oryzias latipes* exposed to different levels of Diazinon

Conc (ppb)	AChE Activity (nmoles substrate hydrolyzed/min/mg protein)	
	Head	Body
Control	91.65±21.03*	120.10±27.25
DMSO	90.78±7.88	107.23±18.67
1	69.23±7.27	117.23±30.01
10	54.1±8.57	93.35±14.36
100	21.65±5.37	31.28±6.07
1000	6.22±0.61	8.26±1.20
5000	5.37±0.67	9.47±4.29

* Mean±SD, Triplicate measurements were performed.

Table 2. Change in acetylcholine esterase activity of *Oryzias latipes* exposed to Diazinon (5 ppm) for different periods

Exposure period (min)	Acetylcholine esterase activity (nmoles substrate hydrolyzed/min/mg protein)	
	Head	Body
0	69.7±7.8*	113.4±8.0
1	69.6±1.8	98.3±6.6
5	64.5±1.1	98.9±16.2
30	56.6±2.2	72.7±9.8
60	35.5±7.1	61.2±13.2
120	14.9±4.7	14.4±1.0
360	8.0±0.2	8.9±0.1

* Mean±SD, Triplicate measurements were performed.

Table 3. Changes in acetylcholine contents of *Oryzias latipes* exposed to different levels of Diazinon

Concentration (ppb)	Acetylcholine contents (nmoles/mg protein)	
	Head	Body
Control	18.53±3.11*	35.48±3.68
DMSO	21.10±2.84	43.26±3.84
1	24.31±5.60	47.05±6.30
10	19.53±1.34	50.45±10.75
100	23.12±3.89	36.42±2.61
1000	33.96±8.00	34.22±0.74
5000	42.30±0.20	39.23±5.96

* Mean±SD, Triplicate measurements were performed.

with 5000 ppb for 240 min in fish brain and body (Table 2).

2. Neurotransmitter contents

Acetylcholine contents in fish head increased by the diazinon concentrations above the 1000 ppb while acetylcholine level in the body was not changed significantly even at 5000 ppb (Table 3). Prolonged exposure of fish to 5000 ppb diazinon did not cause a significant change in acetylcholine contents of head and body as shown in Table 4.

Norepinephrine (NA) contents in head ranged from 2.21 ug/g body wt to 2.56 while its content in the body were present in the range of 1.02 to 1.40 (Table 5). NA content in head part tended to be upregulated with increasing diazinon concentration while NA content in body part was fluctuating with the highest content at 1000 ppb diazinon. Serotonin content of fish treated with the pesticide showed similar pattern with NA and was higher in control and 1000 ppb diazinon groups than 10 or 100 ppb groups. In the

Table 4. Change in acetylcholine contents of *Oryzias latipes* exposed to Diazinon (5 ppm) for different periods

Exposure period (min)	Acetylcholine contents (nmoles/mg protein)	
	Head	Body
0	22.58±5.19*	17.59±2.11
1	22.82±7.43	10.56±0.63
5	38.74±31.86	14.64±4.47
30	23.71±6.68	6.39±6.19
60	17.40±2.19	13.80±8.17
120	22.23±5.03	7.31±0.53
360	31.69±21.56	8.32±1.38

* Mean±SD, Triplicate measurements were performed.

Table 5. Changes in norepinephrine and serotonin contents of *Oryzias latipes* exposed to different levels of Diazinon

Conc. (ppb)	Norepinephrine Conc. (ug/g bw)		Serotonin Conc. (ug/g bw)	
	Head	Body	Head	Body
Control	1.88±0.23*	1.52±0.30	1.42±0.22	1.73±1.28
10	2.41±0.08	1.28±0.14	1.12±0.02	0.37±0.11
100	2.25±0.26	1.45±0.21	1.07±0.09	0.52±0.08
1000	2.57±0.71	1.81±0.51	1.53±0.67	2.42±1.58

* Mean±SD, Triplicate measurements were performed.

Table 6. Change in norepinephrine and serotonin contents of *Oryzias latipes* exposed to Diazinon (5 ppm) for different periods

Exposure period (min)	Norepinephrine Conc. (ug/g bw)		Serotonin Conc. (ug/g bw)	
	Head	Body	Head	Body
Control	2.45±0.38	1.29±0.33	4.85±1.36	2.56±2.49
10	2.21±0.30	1.34±0.29	2.41±0.27	1.90±0.11
30	2.27±0.27	1.06±0.20	4.39±1.29	1.59±0.28
60	2.40±0.70	1.02±0.16	2.77±0.95	1.47±0.64
120	2.66±0.30	1.40±0.59	3.49±2.53	2.47±0.28

* Mean±SD, Triplicate measurements were performed.

fish treated with different levels (0~5000 ppb) of diazinon for 24 hrs, head and body serotonin levels were in the range of 1.1 to 1.6 ug/g and 0.4~2.4 ug/g, respectively. When fish were treated with 5000ppb diazinon for 0 to 120 min, serotonin levels in head and body were 2.4~4.9 and 1.5~2.6 ug/g (Table 6). Serotonin levels in head and body were not affected by exposure time to diazinon.

DISCUSSION

The lethal effects of pesticides on aquatic animals have been extensively studied.[8),9)] Pesticides, however, are generally present in levels much below the concentrations which can directly cause mortality. Thus studies on the effects of pesticides on biochemical parameters are crucial for a comprehensive understanding of chronic toxicity symptoms including behavioral change. Diazinon was reported to induce behavioral change in fish possibly by disturbing acetylcholine esterase activity and/or levels of neurotransmitters.[2),3)] Thus this study was conducted to determine the effect of diazinon on the acetylcholine esterase and neurotransmitter levels in *Oryzias latipes* (killifish).

This study showed that diazinon over 10 ppb inhibited strongly AChE of killifish head and body. The inhibitory activity of diazinon against AChE may be responsible for various behavioral changes observed for fish exposed to the pesticide As neurotranmitters such as acetylcholine, norepinephjrine, and serotonin were reported to regulate behavior and neurological activity, they might also be involved in toxic phenomena seen in killifish held in diazinon-containing water. As pesented, diazinon did not cause a significant change in norepinephrine and serotonin levels in killifish.

However, this result does not exclude the possibility that diazinon affect the local concentration of these neurotransmitters and thereby behavioral pattern of killifish. It has been reported that in goldfish, 1-methyl-4-phenyl-1, 2, 3, 6- tetrahydropyridine (MPTP) administered for 3 consecutive days (10 mg/kg), produced a marked decrease in dopamine (DA) and noradrenaline (NA) levels in telencephalon, diencephalon and medulla oblongata, without affecting the serotonin (5-HT) content.[10)] Further study on the effect of diazinon on enzymes involved in metabolism of neurotransmitters such as tyrosine hydroxylase and monoamine oxidase should aid in elucidating the toxic mechanism of diazinon more accurately.

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