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Acute and long-term genotoxicity of deltamethrin to insulin-like growth factors and growth hormone in rainbow trout

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ABSTRACT

We report here the acute and long-term influences of deltamethrin on the expression of IGF-I, IGF-II and GH-I in rainbow trout muscles. We treated rainbow trouts with different concentrations of deltamethrin (0.25 μg/L, 1 μg/L and 2.5 μg/L) and observed the alterations in mRNA expression levels of IGF-I, IGF-II and GH-I at different time intervals (at 6th, 12th, 24th, 48th, 72nd hours and 30th day). The mRNA levels significantly decreased with increasing deltamethrin concentrations for acute administration. Interestingly, a significant recovery in GH-I expression was seen after the 72nd hour up to 30th day while no significant differences were observed for IGF-I and IGF-II between the same time intervals. Here we demonstrate that deltamethrin exposure decreases the expression of IGF-I, IGF-II and GH-I in rainbow trout which might cause undesirable outcomes not only in growth, but also in development and reproduction.

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1. Introduction

Insulin-like growth factors (IGF-I and -II) are key molecules that regulate numerous cellular processes including proliferation, differentiation, survival and migration. The biological actions of IGFs are mainly mediated through type-1 (IGF-I) receptor on the cell surface, leading to tyrosine phosphorylation of the beta-domain of the receptor and subsequent signaling cascades such as the mitogen-activated protein kinase (MAPK), phosphoinositol-3-kinase and protein kinase B (Akt) signaling pathways (Le Roith et al., 2001; Nakae et al., 2001). In contrast, the type-2 IGF receptor, identified as the cation-independent mannose-6-phosphate receptor, lacks recognizable catalytic domains and presumably works for clearance of IGF-2 (Braulke, 1999; Kajımura and Duan, 2007).

IGF-II is a single chain polypeptide of 67 amino acid residues, that has amino acid homology with IGF-I and proinsulin1 (Humbel, 1990). Both IGF-I and IGF-II bind to the IGF-I receptor with high affinity, which initiates mitogenic and anti-apoptotic responses in the cell (Osborne et al., 1989; Daughaday and Rotwein, 1989; Perdue et al., 1991; Baserga, 1995). Increased expression of IGFs in a variety of cancer cells and various tumours has been reported (Daughaday, 1990). Experimental evidence suggests that IGF-II may act as an autocrine growth factor in the pathogenesis not only of childhood cancers but also of several types of adult cancers (Osborne et al., 1989; Daughaday, 1990; Cullen et al., 1992; Christofori et al., 1994; Rogler et al., 1994; Bates et al., 1995; Fichera et al., 2000).

Growth hormone (GH) is a pleiotropic endocrine factor that is fundamental for somatic growth and development in vertebrates (Chen et al., 1994; Forsyth and Wallis, 2002). The regulatory network for GH is intricate, including both endocrine and environmental factors: the actions of GH are initiated by binding to membrane-bound GH receptors (GHRs) on target tissues (Daughaday, 2000). In vertebrates, the liver is the major target of GH action, and it is also one of the first organs to encounter exogenous metals that are absorbed from diet or environment and transported in the blood (McNulty and Taylor, 1999). In fish, GHRs have been identified in several salmonids (Björnsson et al., 2002) and share many characteristics with the mammalian GHRs, suggesting that the signal transduction and internalization mechanisms of GHR are conserved between fish and mammals (MacDougal et al., 1999; Robertson et al., 2006, Vergani et al., 2009). There are two genes for the growth hormone (GH-I and GH-II) in rainbow trout which have minor differences. However, in both trout and salmon, GH-I mRNA is predominant (Rentier-Delrue et al., 1989).

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The role of IGF-I in regulating postnatal growth and in mediating the growth-promoting effect of GH has been confirmed in many ways not only in mammals but also in fish. Because of the dependence of GH on hepatic IGF-I in realizing this effect, it was previously thought that IGF-I is not important during embryogenesis until it was shown that mice lacking IGF-I had impaired fetal growth and development (Liu et al., 1993).

Pesticides may cause various side effects, e.g. change in DNA structure (Lee and Steinert, 2003), cause of sperm malformations (Mathew et al., 1992), generation of reactive oxygen species (Bagchi et al., 1995), increase in the level of free radicals (Freeman and Crapo, 1982), inhibition of specific enzyme (Ekinci and Beydemir, 2010) influence on antioxidant defense system (Ceyhun et al., 2010; Brian et al., 2005) and act as inducers of heat shock protein (Bagchi et al., 1996) in tissues and cells in different organisms.

Deltamethrin is a non-composite synthetic pyrethroid; a broad spectrum, non-cumulative insecticide; a fast-acting neurotoxic agent with good contact and stomach action and no fumigant action (Fig. 1). Its biological activity is very stable in the environment since it has good residual activity on many insects and most surfaces. Deltamethrin is non-systemic in plants. It is of moderately high toxicity in mammals, and is readily metabolized with rapid loss of toxicity. It has a repellent effect for some insects. It is used in the following concentrations: emulsifiable concentrates (25 g a.i./L); ULV concentrates (4, 5 and 10 g/L); wettable powder (25 and 50 g/kg); dusts (0.5 and 1.0 g/kg); granules (0.5 and 1.0 g/kg); and flowable (25 g a.i./L). Deltamethrin metabolism and excretion have been extensively studied in rats, mice, and cows. The pattern does not vary significantly among these species. Deltamethrin's effect on the parasite is mainly through contact and ingestion. As deltamethrin is extremely lipophilic it easily penetrates the cuticles of insects and acarines. Deltamethrin is metabolized by liver microsomal esterases and oxidases. Hydrolysis initially cleaves the parent compound into two fragments — cyclopropanecarboxylic acid and 3-phenoxybenzyl alcohol. The latter is then oxidised to 3-phenoxybenzoic acid which is the major excretion compound of this moiety. Hydroxylation of a significant portion of this moiety, primarily at the carbon 4′ site but also to some extent at sites 5 and 2, can occur either before or after hydrolysis (Krasnykh et al., 1982).

The relationship between environmental toxicants, IGF expression, and GH regulation has yet to be examined, and the influences of environmental factors on signaling pathways have to be identified. Since favorable or unfavorable conditions for fish growth and development would affect the GH–IGF-I axis, the effects of various chemicals, such as pesticides on expression levels of regulatory components are of great significance and should be well characterized. Here we report on the acute and long-term impacts of deltamethrin on the expression of insulin-like growth factors (IGF-I, IGF-II) and growth hormone-I (GH-I) in rainbow trout (*Oncorhynchus mykiss*).

2. Materials and methods

2.1. Fish husbandry and maintenance

One year old rainbow trout (*O. mykiss*, Salmonidae) (mixed sexes) were obtained from the Fisheries Department of the Agricultural Faculty at Atatürk University in Erzurum and weighed of 150 ± 10 g in autumn 2009. They were fed twice a day with a commercial pelleted trout feed at a daily ration of 1% of their wet body mass during the study. Trout feed was purchased from Pınar Yem Company, İzmir-Turkey. Feed was given by hand. Fish treatments were conducted according to the Applied Research Ethics National Association (ARENA) (2002).

Prior to experiments, fish in each group were kept in 1 × 1.2 m (wide-deep) fiber-glass tanks for adaptation during one month. Aeration was provided along the experiments. The average water temperature was 9 °C during the tests. The water quality parameters were analyzed in the Laboratory of Erzurum Hygiene Institute. Fiber-glass tanks contained fifteen fish in 600 L water. The average dissolved oxygen level was adjusted to 8.8 ppm and maintained by means of a ventilation system. One tank was used as control, and did not contain pesticide while others were added 0.25 μg/L, 1 μg/L and 2.5 μg/L deltamethrin, respectively. The muscle samples were collected after the pesticide treatment from each group fish at 6, 12, 24, 48, 72 h and 30 days. All fish samples were frozen with liquid nitrogen and stored at −86 °C until RNA isolation.

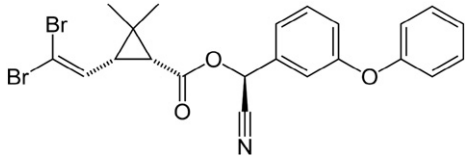


Fig. 1. Molecular structure of deltamethrin.

2.2. RNA isolation and cDNA synthesis

Total RNA was isolated from 500 mg of frozen white muscle tissues, between the dorsal fin and lateral line, of both deltamethrin treated and control fish using TRIzol reagent. RNA was treated with DNase in order to avoid genomic contamination. RNA concentrations and quality were verified by means of spectrophotometer (Nanodrop) and RNA gel electrophoresis, respectively. Following isolation, cDNA synthesis was performed using the ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis Kit (Invitrogen) according to the manufacturer's protocol. All cDNA was stored at −20 °C until use.

2.3. TaqMan probe and primer design

Primers and TaqMan probes were designed in Primer3 software (v. 0.4.0) (http://frodo.wi.mit.edu/) using rainbow trout IGF-I (GenBank accession number M95183.1), IGF-II (M95184.1) and GH-I (NM_001124689.1) sequences and BLASTed to ensure correct mRNA sequences. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as the reference gene (NM_001124246). In order to perform multiplex Real-time PCR, the TaqMan probe of the housekeeping gene was conjugated with Cy5/Blackhole Quencher 2, the fluorophore and quencher molecules, whereas the probes for target genes were conjugated with FAM/TAMRA. The primer and probe sequences are provided in Table 1.

2.4. Real-time PCR

Quantification of gene expression by real-time PCR analysis was performed using a thermal cycler Stratagene MxPro3000. The real-time PCR was carried out in a reaction volume of 50 μL containing template DNA, 900 nM of both target and reference forward and reverse primers, 250 nM both target and reference TaqMan probes, and 25 μL FastStart TaqMan Probe Master (Applied Biosystems) which consists of AmpliTaq Gold DNA Polymerase, AmpErase uracil N-glycosylase (UNG), dNTP with dUTP, and optimized buffer component. Amplification and detection of the samples and the standards were performed using the following thermal cycling conditions: 50 °C for 2 min for activation of optical AmpErase UNG enzyme, 95 °C for 10 min as hot start to activate AmpliTaq Gold DNA polymerase followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. Real-time PCR data were analyzed using the efficiency $(e)^{(-\Delta\Delta Ct)}$ method (Pfaffl, 2001), which is used to determine mean fold changes in gene expression against control group and housekeeping gene GAPDH. Analytical sensitivity was confirmed by running standard curves. Amplification efficiency (e) was calculated based on the slopes of the

Table 1
Sequence, amplification length, genbank accession number of primers and probes used for real-time PCR.

Primers and probes	Sequence (5'-3')	Amplification length (bp)	GenBank accession number
GH-I Forward	AATGGTCAGAAATGCCAACC	201	NM_001124689.1
GH-I Reverse	AAGCAAGCCAACAACTCGTAG		
GH-I Probe	^{FAM}-CATCAACCTGCTCATCACGGGG-^{TAMRA}		
IGF-I Forward	ATGTGCTGTGTCTCCTGTACCC	149	M95183.1
IGF-I Reverse	TAAAAGCCTCTCTCTCCACACA		
IGF-I Probe	^{FAM}-TAACCCTGACTTCGGCGGCA-^{TAMRA}		
IGF-II Forward	GAAGGTCAAGATGATGTCTTCG	108	M95184.1
IGF-II Reverse	AGTTCTCCTCCACATAGCGTTT		
IGF-II Probe	^{FAM}-TCGAGTGCTGGTCATTGCGC-^{TAMRA}		
GAPDH Forward	ATCAAAGGGGCTGTCAAGAA	106	NM_001124246
GAPDH Reverse	AGGAGTGGGTGTCTCCAATG		
GAPDH Probe	^{Cy5}-CGCCGAAGGACCCATGAAGG-^{BQ2}		

curves (slope) using the formula $e = 10^{(-1/slope)}$ (Pfaffl, 2001), and the slope value via Stratagene MxPro3000 software.

2.5. Statistical analyses

All experiments were triplicated. Presented data show differences between control and treated groups. Following arc-sine transformations, one-way ANOVA and Duncan's multiple range test (SPSS 17.0) was used to determine the toxic effects of deltamethrin and $P<0.05$ was considered statistically significant.

3. Results

In deltamethrin treated rainbow trouts, sampling was managed until day 30 for 0.25 μg/L dosage. However, it was able to be managed up to 48 h for 1 μg/L dosage and up to 24 h for 2.5 μg/L dosage. Fish exposed to 1 μg/L and 2.5 μg/L deltamethrin died at 72 and 48 h, respectively (Table 2).

GH-I, IGF-I and IGF-II expressions gradually decreased depending on deltamethrin exposure time at 6, 12, 24, 48 and 72 h. When acute and chronic exposure data were compared, a significant increase in GH-I expression is clearly seen indicating the recovery of fish from the 72nd hour up to day 30. However, no significant differences were observed in the expression of IGF-I and IGF-II between 72 h and day 30. In acute exposure, the GH-I mRNA expression level significantly decreased beginning from the 6th hour whereas those of IGF-I and IGF-II started to decrease at the 24th hour together with increasing deltamethrin concentrations (0.25 μg/L, 1 μg/L and 2.5 μg/L). In overall results obtained, the time-dependent changes in acute and chronic effects of deltamethrin are similar for GH-I, IGF-I and IGF-II levels (Table 2).

4. Discussion

The investigation of the modulations in expression levels of insulin-like growth factors and growth hormone has become of particular interest for toxicology researchers. For instance, Nieves-Puigdoller et al. (2007) reported that exposure to hexazinone (HEX) and atrazine (ATZ), highly mobile and widely used herbicides along rivers in the United States, reduced feeding after 10 days of exposure and had an impaired growth rate in Atlantic salmon. They stated that HEX and ATZ at 10 μg/L exposure had no effect on plasma levels of cortisol, growth hormone (GH), and insulin growth factor I (IGF-I).

Eder et al. (2008) demonstrated that treatment of juvenile Chinook salmon with chlorpyrifos (CP) and esfenvalerate (EV) led to significantly decreased IGF-1 transcription in the spleen on days 20 and 60, whereas a short-term increase was seen after CP exposure (day 4).

Very et al. (2008) used rainbow trout to assess the effects of somatostatins on the production of IGF-I. Somatostatin-14 (SS-14-I) implantation for 15 days significantly reduced steady-state levels of IGF-I mRNA in the liver and lowered IGF-I concentration in plasma compared to control animals. The direct effects of somatostatins were examined on hepatocytes incubated *in vitro*. SS-14-I inhibited basal and GH-stimulated IGF-I mRNA expression. SS-14-I inhibition of GH-stimulated IGF-I expression was concentration- and time-dependent.

Luckenbach et al. (2007) aimed to examine the effects of temperature on growth and establish whether IGF-I may mediate growth

Table 2
Relative mRNA expression levels of GH-I, IGF-I, IGF-II in the muscles of rainbow trout exposed to different deltamethrin dosages. GAPDH was used as the reference gene.

Genes	Conc. (μg/L)	Acute effect					Chronic effect
		Hours					Days
		6	12	24	48	72	30
GH-I	0.25	0.41 ± 0.01^{aA}	0.04 ± 0.01^{dA}	0.07 ± 0.01^{cA}	0.03 ± 0.01^{dA}	0.01 ± 0.01^{e}	0.35 ± 0.01^{b}
	1	0.13 ± 0.01^{aB}	0.03 ± 0.00^{bB}	0.02 ± 0.00^{bB}	0.03 ± 0.00^{bA}	Fish died	–
	2.5	0.04 ± 0.01^{aC}	0.01 ± 0.00^{bC}	0.01 ± 0.00^{bB}	Fish died	–	–
IGF-I	0.25	10.93 ± 1.50^{aA}	5.90 ± 1.48^{bA}	5.75 ± 1.98^{bA}	0.23 ± 0.05^{cA}	0.17 ± 0.03^{c}	1.70 ± 0.19^{c}
	1	9.35 ± 4.09^{aA}	7.81 ± 2.80^{abA}	4.63 ± 1.55^{abAB}	0.28 ± 0.03^{bA}	Fish died	–
	2.5	17.94 ± 4.21^{aA}	11.68 ± 5.11^{abA}	0.47 ± 0.15^{bB}	Fish died	–	–
IGF-II	0.25	5.48 ± 1.75^{aA}	2.83 ± 0.47^{bA}	2.04 ± 0.37^{bA}	1.21 ± 0.27^{bA}	0.42 ± 0.15^{b}	0.28 ± 0.12^{b}
	1	4.91 ± 1.53^{aA}	5.00 ± 1.63^{aA}	1.01 ± 0.29^{bB}	0.30 ± 0.03^{bB}	Fish died	–
	2.5	1.79 ± 0.35^{abA}	3.29 ± 0.86^{aA}	0.47 ± 0.16^{bB}	Fish died	–	–

$P<0.05$: significant.
a, b, c, d = statistical signifigance between each values in the rows.
A, B, C = statistical signifigance between each values in the columns.
Statistical analysis was performed by means of One-Way ANOVA Duncan's multiple range test (SPSS 17.0 program).

at different temperatures in southern flounder, *Paralichthys lethostigma*. In two experiments, juvenile flounder were reared at 23 and 28 °C and growth was monitored for either 117 or 197 days. They reported that growth was similar across treatments in both experiments until fish reached approximately 100 mm total length. Body size then diverged with fish at 23 °C ultimately growing 65–83% larger than those at 28 °C. Muscle IGF-I mRNA, plasma IGF-I, and hepatosomatic index (HSI) were significantly higher in flounder at 23 °C, whereas hepatic IGF-I mRNA abundance did not differ with treatment. Muscle IGF-I mRNA was correlated with HSI, while plasma IGF-I was correlated with body size, hepatic IGF-I mRNA, and HSI. Their results demonstrated a strong effect of temperature on flounder growth and show that temperature-induced variation in growth is associated with differences in systemic IGF-I and local (i.e., muscle) IGF-I mRNA levels.

In another study, administration of dibutyryl cyclic AMP and cortisol produced a significant elevation of GH mRNA levels in rainbow trout leucocytes. GH1 and GH2 mRNA levels showed similarities in responses to those factors. A dose-depending elevation of GH gene expression was observed in leucocytes treated with cortisol at below 200 nM, however, cortisol had no effect at 2000 nM. The results thus showed that, at the low levels, GH gene expression in trout leucocytes is regulated by cortisol, which has been known as a regulatory factor of GH gene expression in pituitary cells, and suggest a physiological significance of paracrine GH produced in the fish immune system (Yada et al., 2005).

Poppingaa et al. (2007) examined the role of somatostatin-14 (SS-14), a potent inhibitor of GH, on the GH–IGF-1 axis and seawater adaptation. In the study, juvenile rainbow trout (*O. mykiss*) were injected intraperitoneally with SS-14 or saline and transferred to 20 ppt seawater. Seawater increased hepatic mRNA levels of GH receptor 1 (GHR 1; 239%), GHR 2 (48%), and IGF-1 (103%) in control fish 12 h after transfer. Levels of GHR 1 (155%), GHR 2 (121%), IGF-1 (200%), IGF-1 receptor A (IGFR1A; 62%), and IGFR1B (157%) increased in the gills of control fish 12 h after transfer. They reported that SS-14 abolished or attenuated seawater-induced changes in the expression of GHR, IGF-1, and IGFR mRNAs in the liver and gill. The results indicated that SS-14 reduces seawater adaptability by inhibiting the GH–IGF-1 axis.

The presence of IGF-I in many types of brain injuries makes this peptide a likely participant in reactive responses to damage (Dávila and Torres-Aleman, 2008). Indeed, IGF-I protects against neuronal insults (Trejo et al., 2004) and is required for recovery after injury (Fernandez et al., 1999). However, under experimental conditions recreating oxidative stress, IGF-I neuroprotection fails (Wu et al., 2006; Zhong and Lee, 2007). In this context, reactive oxygen species (ROS) were reported to inactivate IGF-I receptor function through abnormal glycation (Wu et al., 2006). This process could reflect a nonspecific generalized protein oxidation after prolonged exposure to ROS (Wu et al., 2006).

However, IGF-I signaling is probably targeted by ROS in a more specific way. Thus, several mediators of the IGF-I/insulin signaling cascade are modulated by a redox state under physiological conditions (Lee et al., 2002; Leslie, 2006). IGF-I likely modulates the generation of ROS by neurons through regulation of neuronal metabolism (Sonntag et al., 2006). Additional support for this possibility is that ROS specifically interferes with insulin signaling at different steps (Hansen et al., 1999; Houstis et al., 2006), and insulin and IGF-I share the same or very similar intracellular pathways. Because the IGF-IR/AKT pathway is a wide-spectrum neuroprotective route, its attenuation may be involved in cell death by oxidative stress. In the previous work this possibility was explored in detail. It was found that oxidative stress elicits neuronal death through activation of FOXO, a transcription factor whose activity is inhibited by insulin-like growth factor I (IGF-I), by a dual process that involves timed activation of stress kinases and abrogation of IGF-I neuroprotection (Dávila and Torres-Aleman, 2008).

Sayeed et al. (2003) investigated the effect of deltamethrin (0.75 μg/L) on antioxidants in a freshwater fish, *Channa punctatus*, using standard laboratory conditions. They observed that a single exposure for 48 h caused induction of various antioxidant enzymes and nonenzymatic antioxidants in the kidney and liver. The induction of these antioxidants was not very prominent in gills. In fact, certain antioxidants were depleted in gills. Catalase activity was decreased in all tissues. Deltamethrin also induced lipid peroxidation in all the tissues, gills showing the highest levels. Glutathione, which is an established nonenzymatic antioxidant in fish, was significantly ($P<0.001$) increased in all the tissues. Ascorbic acid content increased in the kidney and liver while it decreased in gills. The findings of the investigation showed that deltamethrin has oxidative stress-inducing potential in fish, and gills are the most sensitive organs.

The impact of commonly used pesticides, endosulfan and deltamethrin, on the molecular stress level in black tiger shrimp *Penaeus monodon*, was investigated using classical oxidative stress biomarkers, protein carbonylation profiles, and levels of heat shock proteins. Results showed that 4 days exposure to 0.1 μg/L deltamethrin significantly ($P<0.05$) increased lipid peroxidation (LPO) level in gills (Dorts et al., 2009).

Modulatory effect of copper pre-exposure (10 ppb) on deltamethrin (0.75 μg/L) induced oxidative stress was investigated in freshwater fish *C. punctatus*. Nonenzymatic antioxidants were studied as biomarkers of exposure to deltamethrin and possible protection afforded by copper pre-exposure. Glutathione levels were reduced significantly ($P<0.05$) in liver of copper-acclimatized deltamethrin-exposed group when compared with deltamethrin-exposed groups. The total thiol levels of the copper-acclimatized deltamethrin-exposed group was significantly lower ($P<0.01$) in the liver when compared with deltamethrin-exposed group, while non-protein thiol levels recorded a significant ($P<0.01$) increase in the liver of the copper-acclimatized deltamethrin-exposed group when compared with the deltamethrin-exposed group. The lipid peroxidation levels of copper-acclimatized deltamethrin-exposed groups were significantly lowered ($P<0.01$) in the liver when compared with deltamethrin-exposed group (Parvez and Raisuddin, 2006).

In addition, we demonstrated in our previous study that deltamethrin inhibits antioxidant enzymes and elevates the expression levels of heat shock protein 70 (Ceyhun et al., 2010) and the current study reports the negative effects of the pesticide on the expression of IGF-I, IGF-II and GH. Therefore, combining the literature work with our previous and current study, we speculate that decrease in the expression of these genes might be resulted through the formation of oxidant agents in response to deltamethrin exposure.

5. Conclusion

The growth hormone–insulin-like growth factor (GH–IGF) axis has gained considerable focus over recent years. One cause of this increased interest is due to a correlation of age-related decline in plasma GH/IGF levels with age-related degenerative processes, and it has led to the prescribing of GH replacement therapy by some practitioners (Scarth, 2006). On the other hand, however, research has also focused on the pro-carcinogenic effects of high GH–IGF levels, providing strong impetus for finding regimes that reduce its activity. Whereas the effects of GH/IGF activity on the action of xenobiotic-metabolizing enzyme systems is reasonably well appreciated, the effects of xenobiotic exposure on the GH–IGF axis has not received substantial review. Relevant xenobiotics are derived from pharmaceutical, nutraceutical and environmental exposure, and many of the mechanisms involved are highly complex in nature, not easily predictable from existing *in vitro* tests and do not always predict well from in vivo animal models. Molecular determination of how specific genes such as GH, IGF-I and IGF-II are affected in positive or negative ways is thus very important for both molecular biological purposes and fishery products cultivation. In this respect, our study makes good contributions to literature work evidencing that expressions of GH-I, IGF-I and IGF-II significantly decrease in response to deltamethrin

exposure which might cause undesirable consequences not only in growth, but also in development and reproduction.

References

Bagchi, D., Bagchi, M., Hassoun, E., Stohs, S.J., 1995. In vitro and in vivo generation of reactiveoxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology 104, 129–140.

Bagchi, D., Bhattacharya, G., Stohs, S.J., 1996. In vitro and in vivo induction of heat shock (stress) protein (Hsp) gene expression by selected pesticides. Toxicology 112, 57–66.

Baserga, R., 1995. The insulin-like growth factor-I receptor: a key to tumor growth. Cancer Res. 55, 249–252.

Bates, P., Fisher, R., Ward, A., Richardson, L., Hill, D.J., Graham, C.F., 1995. Mammary cancer in transgenic mice expressing insulin-like growth factor II (IGF-II). Br. J. Cancer 72, 1189–1193.

Björnsson, B.T., Johansson, V., Benedet, S., Einarsdottir, I.E., Hildahl, J., Agustsson, T., Jönsson, E., 2002. Growth hormone endocrinology of salmonids: regulatory mechanism and mode of actions. Fish Physiol. Biochem. 27, 227–242.

Braulke, T., 1999. Type-2 IGF receptor: a multi-ligand binding protein. Horm. Metab. Res. 31, 242–246.

Brian, K.B., Donna, W.L., Deborah, A.C., Lisa, A.O., 2005. Modulation of antioxidant defense systems by the environmental pesticide maneb in dopaminergic cells. NeuroToxicology 26, 63–75.

Ceyhun, S.B., Senturk, M., Ekinci, D., Erdogan, O., Ciltas, A., Kocaman, M., 2010. Deltamethrin attenuates antioxidant defense system and induces expression of heat shock protein 70 in rainbow trout. Comp. Biochem. Physiol. C 152, 215–223.

Chen, W.Y., Chen, N.Y., Yun, J., Wagner, T.E., Kopchick, J.J., 1994. In vitro and in vivo studies of antagonistic effects of human growth hormone analogs. J. Biol. Chem. 269, 15892–15897.

Christofori, G., Naik, P., Hanahan, D., 1994. A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. Nature 369, 414–418.

Cullen, K.J., Lippman, M.E., Chow, D., Hill, S., Rosen, N., Zwiebel, J.A., 1992. Insulin-like growth factor-II overexpression in MCF-7 cells induces phenotypic changes associated with malignant progression. Mol. Endocrinol. 6, 91–100.

Daughaday, W.H., 1990. The possible autocrine/paracrine and endocrine roles of insulin-like growth factors of human tumors. Endocrinology 127, 1–4.

Daughaday, W.H., 2000. Growth hormone axis overview—somatomedin hypothesis. Pediatr. Nephrol. 14, 537–540.

Daughaday, W.H., Rotwein, P., 1989. Insulin-like growth factors 1 and 2, peptide messenger ribonucleic acid, gene structures and serum and tissue concentrations. Endocr. Rev. 10, 68–91.

Dávila, D., Torres-Aleman, I., 2008. Neuronal Death by Oxidative stress ınvolves activation of FOXO3 through a two-arm pathway that activates stres kinases and attenuates ınsulin-like growth factor 1 signaling. Mol. Biol. Cell 19, 2014–2025.

Dorts, J., Silvestre, F., Tu, H.T., Tyberghein, A., Phuong, N.T., Kestemont, P., 2009. Oxidative stress, protein carbonylation and heat shock proteins in the black tiger shrimp, *Penaeus monodon*, following exposure to endosulfan and deltamethrin. Environ. Toxicol. Pharmacol. 28, 302–310.

Eder, K.J., Clifford, M.A., Hedrick, R.P., Köhler, Heinz-R, Werner, I., 2008. Expression of immune-regulatory genes in juvenile Chinook salmon following exposure to pesticides and infectious hematopoietic necrosis virus (IHNV). Fish Shellfish Immunol. 25, 508–516.

Ekinci, D., Beydemir, S., 2010. Risk assessment of pesticides and fungicides for acid–base regulation and salt transport in rainbow trout tissues. Pesticide Biochem. Physiol. 97, 66–70.

Fernandez, A.M., Gonzalez de la Vega, A.G., Planas, B., Torres-Aleman, I., 1999. Neuroprotective actions of peripherally administered insulin-like growth factor I in the injured olivo-cerebellar pathway. Eur. J. NeuroSci. 11, 2019–2030.

Fichera, E., Liang, S., Xu, Z., Guo, N., Mineo, R., Fujita-Yamaguchi, Y., 2000. A quantitative reverse transcription and polymerase chain reaction assay for human IGF-II allows direct comparison of IGF-II mRNA levels in cancerous breast, bladder, and prostate tissues. Growth Horm. IGF Res. 10, 61–70.

Forsyth, I.A., Wallis, M., 2002. Growth hormone and prolactin-molecular and functional evolution. J. Mammary Gland Biol. Neoplasia 7, 291–312.

Freeman, B., Crapo, J., 1982. Biology of disease. Free radicals and tissue injury. Lab. Invest. 47, 412–426.

Hansen, L.L., Ikeda, Y., Olsen, G.S., Busch, A.K., Mosthaf, L., 1999. Insulin signaling is inhibited by micromolar concentrations of H(2)O(2). Evidence for a role of H(2)O(2) in tumor necrosis factor alpha-mediated insulin resistance. J. Biol. Chem. 274, 25078–25084.

Houstis, N., Rosen, E.D., Lander, E.S., 2006. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 440, 944–948.

Humbel, R.E., 1990. Insulin-like growth factors I and II. Eur. J. Biochem. 190, 445–462.

Kajımura, S., Duan, C., 2007. Insulin-like growth factor-binding protein-1: an evolutionarily conserved fine tuner of insulin-like growth factor action under catabolic and stressful conditions. J. Fish. Biol. 71, 309–325 (Supplement C).

Krasnykh, A.A., Shustov, V.S., Zelenina, M.F., 1982. Experience with the chromatographic determination of selekron in water, soil and plants. Gig. Sanit. 2, 76–77.

Le Roith, D., Bondy, C., Yakar, S., Liu, J.L., Butler, A., 2001. The somatomedin hypothesis. Endocr. Rev. 22, 53–74.

Lee, S.R., Yang, K.S., Kwon, J., Lee, C., Jeong, W., Rhee, S.G., 2002. Reversible inactivation of the tumor suppressor PTEN by H2O2. J. Biol. Chem. 277, 20336–20342.

Lee, R.F., Steinert, S., 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat. Res. 544, 43–64.

Leslie, N.R., 2006. The redox regulation of PI 3-kinase-dependent signaling. Antioxid. Redox Signal. 8, 1765–1774.

Liu, J.P., Baker, J., Perkins, A.S., Robertson, E.J., Efstratiadis, A., 1993. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75, 59–72.

Luckenbach, J.A., Murashigea, R., Danielsa, H.V., Godwina, J., Borski, R.J., 2007. Temperature affects insulin-like growth factor I and growth of juvenile southern flounder, *Paralichthys lethostigma*. Comp. Biochem. Physiol. A 146, 95–104.

MacDougal, K.C., Mericko, P.A., Burnett, K.G., 1999. Antigen receptor-mediated activation of extracellular related kinase (ERK) in B lymphocytes of teleost fishes. Dev. Comp. Immunol. 23, 221–230.

Mathew, G., Vijayalaxmi, K.K., Rahiman, M.A., 1992. Methyl parathion-induced sperm shape abnormalities in mouse. Mutat. Res. 280, 169–173.

McNulty, T.J., Taylor, C.W., 1999. Extracellular heavy-metal ions stimulate Ca2+ mobilization in hepatocytes. Biochem. J. 339, 555–561.

Nakae, J., Kido, Y., Accili, D., 2001. Distinct and overlapping functions of insulin and IGF-I receptors. Endocr. Rev. 22, 818–835.

Nieves-Puigdoller, K., Björnsson, B.T., McCormick, S.D., 2007. Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. Aquat. Toxicol. 84, 27–37.

Osborne, C.K., Coronado, E.B., Kitten, L.J., Arteaga, C.I., Fuqua, S.A.W., Ramasharma, K., Marshall, M., Li, C.H., 1989. Insulin-like growth factor-II (IGF-II): a potential autocrine/paracrine growth factor for human breast cancer acting via the IGF-I receptor. Mol. Endocrinol. 3, 1701–1709.

Parvez, S., Raisuddin, S., 2006. Copper modulates non-enzymatic antioxidants in the freshwater fish Channa punctata (Bloch) exposed to deltamethrin. Chemosphere 62, 1324–1332.

Perdue, J.F., LeBon, T.R., Kato, J., Hampton, B., Fujita-Yamaguchi, Y., 1991. Binding specificities and transducing function of the different molecular weight forms of insulin-like growth factor-II (IGF-II) on IGF-I receptors. Endocrinology 129, 3101–3108.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acid Res. 29, 2003–2007.

Poppingaa, J., Kittilsona, J., McCormick, S.D., Sheridan, M.A., 2007. Effects of somatostatin on the growth hormone-insulin-like growth factor axis and seawater adaptation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 273, 312–319.

Rentier-Delrue, F., Swennen, D., Mercier, L., Lion, M., Benrubi, O., Martial, J.A., 1989. Molecular cloning and characterization of two forms of trout growth hormone cDNA: expression and secretion of tGH-II by *Escherichia coli*. DNA 8, 109–117.

Robertson, K., Kopchick, J.J., Liu, J.L., 2006. Growth hormone receptor gene deficiency causes delayed insulin responsiveness in skeletal muscles without affecting compensatory islet cell overgrowth in obese mice. Am. J. Physiol. Endocrinol. Metab. 291, 491–498.

Rogler, C.E., Yang, D., Rossetti, L., Donohoe, J., Alt, E., Chang, C.J., Rosenfeld, R., Neely, K., Hintz, R., 1994. Altered body composition and increased frequency of diverse malignancies in insulin-like growth factor-II transgenic mice. J. Biol. Chem. 269, 13779–13784.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R., Raisuddin, S., 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicol. Environ. Saf. 56, 295–301.

Scarth, J.P., 2006. Modulation of the growth hormone—insulin-like growth factor (GH–IGF) axis by pharmaceutical, nutraceutical and environmental xenobiotics: An emerging role for xenobiotic-metabolizing enzymes and the transcription factors regulating their expression. A review. Xenobiotica 36, 119–218.

Sonntag, W.E., Bennett, C., Ingram, R., Donahue, A., Ingraham, J., Chen, H., Moore, T., Brunso-Bechtold, J.K., Riddle, D., 2006. Growth hormone and IGF-I modulate local cerebral glucose utilization and ATP levels in a model of adult-onset growth hormone deficiency. Am. J. Physiol. Endocrinol. Metab. 291, E604–E610.

Trejo, J.L., Carro, E., Garcia-Galloway, E., Torres-Aleman, I., 2004. Role of insulin-like growth factor I signaling in neurodegenerative diseases. J. Mol. Med. 82, 156–162.

Vergani, L., Lanza, C., Scarabelli, L., Canesi, L., Gallo, G., 2009. Heavy metal and growth hormone pathways in metallothionein regulation in fish RTH-149 cell line. Comp. Biochem. Physiol. C 149, 572–580.

Very, N.M., Kittilsona, J.D., Kleina, S.E., Sheridan, M.A., 2008. Somatostatin inhibits basal and growth hormone-stimulated hepatic insulin-like growth factor-I production. Mol. Cell. Endocrinol. 281, 19–26.

Wu, D.C., Re, D.B., Nagai, M., Ischiropoulos, H., Przedborski, S., 2006. The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. Proc. Natl. Acad. Sci. U.S.A. 103, 12132–12137.

Yada, T., Muto, K., Azuma, T., Hyodo, S., Schreck, C.B., 2005. Cortisol stimulates growth hormone gene expression in rainbow trout leucocytes in vitro. Gen. Comp. Endocrinol. 142, 248–255.

Zhong, J., Lee, W.H., 2007. Hydrogen peroxide attenuates insulin-like growth factor-1 neuroprotective effect, prevented by minocycline. Neurochem. Int. 51, 398–404.