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Effects of 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) on Hsp70 Gene Expression in Rainbow Trout

Orhan Erdogan[1]*, Muhammed Atamanalp[1], Turgay Sisman[2], Ercument Aksakal[1], and Gonca Alak[1]

[1] *Department of Aquaculture, Agriculture Faculty, Ataturk University, 25240 Erzurum, Turkey*

[2] *Department of Biology, Arts and Science Faculty, Ataturk University, 25240 Erzurum, Turkey*

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Abstract

2,2-Dichlorovinyl dimethyl phosphate (DDVP) is used to control insects on crops, household, and stored products, and treat external parasitic infections in farmed fish, livestock, and domestic animals. Ectoparasitic copepods can cause severe skin damage in fish that may lead to death through osmoregulatory failure or infection by opportunistic pathogens. There is considerable uncertainty about whether or not DDVP is implicated in cancer, and the wider environmental consequences of its use. In general, and specifically in developing countries and fish farming, less hazardous alternatives are available. The present experiment studied the effects of DDVP at a daily dose of 1.6 mg/l for 21 days on the expression of the heat shock protein (Hsp) 70 gene in rainbow trout (*Oncorhynchus mykiss*). Hsp70 from control and DDVP-exposed fish was amplified for 20-40 PCR cycling. After the fortieth PCR cycle, the Hsp70 level in mRNA was very low in the control fish and very high in the DDVP-exposed fish, with a statistical difference of $p<0.01$.

Introduction

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate; DDVP) is a relatively non-persistent organophosphate compound that undergoes quick and complete hydrolysis in most environments and is rapidly degraded by mammalian metabolism (WHO, 1989). It is used to control insects on crops, household, and stored products, and to treat external parasite infections of farmed fish, livestock, and domestic animals. Ectoparasitic copepods can result in severe skin damage in affected fish, which may lead to death through osmoregulatory failure or infection by opportunistic pathogens (Bruno et al., 1990). The

* Corresponding author. Tel: +90-442-2313482, fax: +90-442-2360958,
e-mail: oerdogan@atauni.edu.tr

main mechanism for controlling such infestations is by immersing the fish in a DDVP bath (McHenery et al., 1997). Infested fish are bathed in dichlorvos in cages surrounded by tarpaulins (Rae, 1979). After treatment, the tarpaulins are removed and the treatment water containing the dichlorvos is released into the surrounding water where the compound is rapidly dispersed and diluted (Turrell, 1990; Wells et al., 1990; Dobson and Tack, 1991).

DDVP kills crustaceans by inhibiting their acetylcholinesterase (AChE) activity. It is accepted that dichlorvos is dangerous to a number of aquatic species and that the discharge of dichlorvos into water should be reduced. Dichlorvos can inhibit cholinesterase levels in humans, which may lead to short or long-term neurotoxic effects (Howard, 1991). Although it has been used for some forty years, considerable uncertainties remain about whether or not DDVP is implicated in cancer and the wider environmental consequences of its use.

In general, and specifically in developing countries and in fish farming, less hazardous alternatives are available. There is evidence that dichlorvos is mutagenic in bacteria, fungi, and mammalian cells *in vitro*, but there is no evidence for mutagenicity in whole animals when it is rapidly degraded. Dichlorvos does not significantly bioaccumulate in fish (Howard, 1991). The present study investigated the effects of DDVP exposure on Hsp70 gene expression in rainbow trout.

Materials and Methods

Fish and DDVP. Forty-eight one-year-old rainbow trout (*Oncorhynchus mykiss,* Walbaum; mean mass 140±25 g) were obtained from the Fisheries Department of the Agricultural Faculty at Ataturk University in Erzurum. Twelve fish were stocked in each of four tanks with aerated water at a constant water flow of 0.5 l/min/kg, an average temperature of 9°C, 9 ppm dissolved oxygen, pH 7.8, and total hardness of 102 mg $CaCO_3$. The fish were fed a commercial pellet diet at a daily ration of 1% of their wet body mass. Feed was given by hand. After an adaptation period of 14 days, two tanks of fish were exposed to one dose of 1.6 mg DDVP per liter water per day for 21 days. The remaining two tanks served as an unexposed control.

RNA and cDNA synthesis. Fast-frozen skeletal muscle tissue of each sample was used for purification of total RNA. Total RNA was extracted using TRIzol® Reagent (Invitrogen). First-strand cDNA was synthesized using Super Script III Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol.

Quantitative reverse transcription polymerase chain reaction (RT-PCR). For PCR reaction, we used ~100 ng template, 1x PCR buffer (1.5 mM $MgCl_2$), 200 µM of each dNTP, 0.2 µM gene-specific forward (5´-TGCACCTAGGTTTTCATAGAAT-3´) and reverse (5´-ATGGAGGTGTAGAAGTCGATGC-3´) primers, and 2.5 units of Taq DNA polymerase to produce a total reaction volume of 15 µl. Thermal cycling conditions included an initial activation at 94°C for 3 min, 20-40 PCR cycles of 94°C for 30 s, 62.5°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were submitted to electrophoresis on 1% agarose gel. A distinct band estimated at ~954 nucleotides was generated. The same method was performed for the actin gene using primers actinF (5'-TGGGGCAGTATGGCTTGTATG-3') and actinR (5'-CTCTGGCACCCTAATCACCTCT-3') as the internal control (Ojimaa et al., 2005). PCR with primers actinF and actinR was performed using the same method except that the PCR cycles were only forty PCR cycles. Amplified products were quantified using ImageJ 1.37c (http://rsb.info.nih.gov/ij/).

Results

After exposure to 1.6 mg/l DDVP once every 24 hours for 21 days, Hsp70 from mRNA of treated fish was very low in control fish and very high in fish exposed to DDVP (Fig. 1). The Hsp70 levels were plotted using gel density after the fortieth PCR cycle against β-actin as the control (Fig. 2). The quantitative mRNA level in fish exposed to DDVP was more than five times higher than in the control with a statistical difference of $p<0.01$.

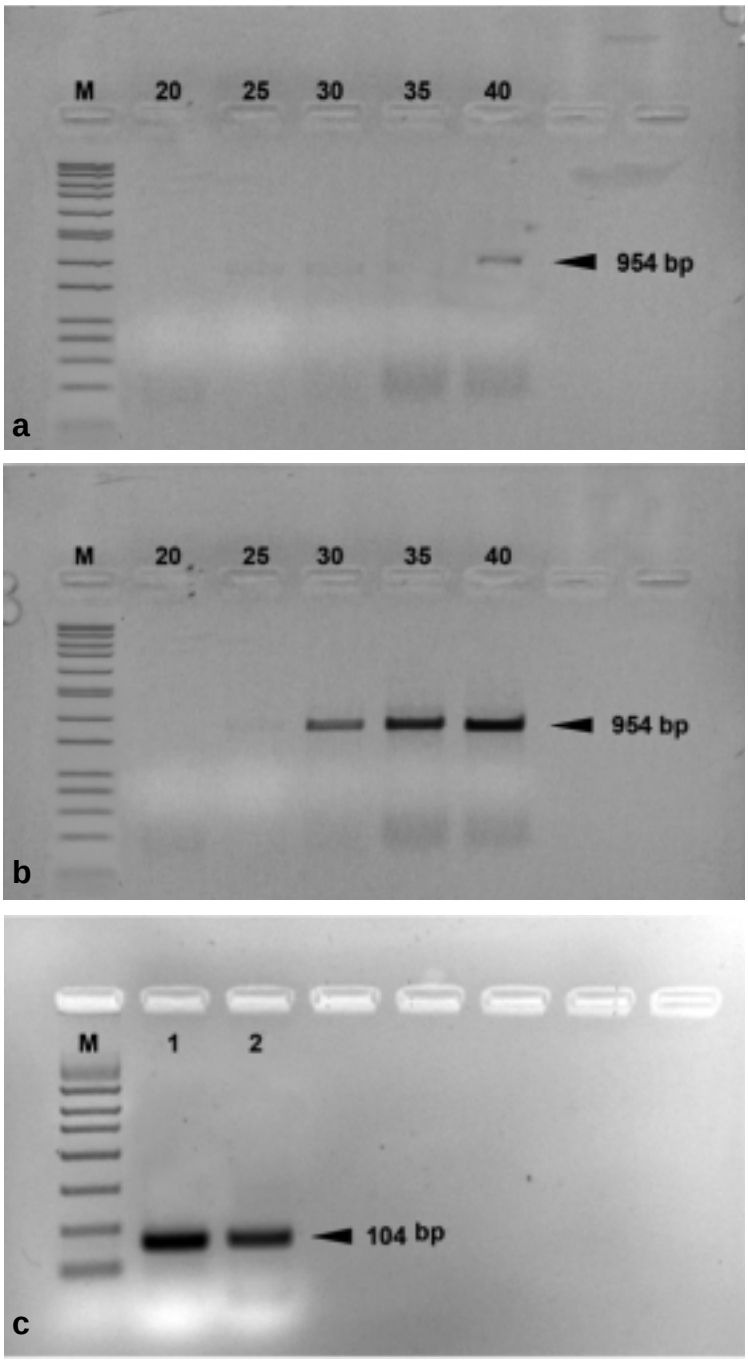

Fig. 1. Hsp70 gene agarose gel patterns of (a) control fish and (b) fish exposed to a daily dose of 1.6 mg DDVP per liter water after 20-40 PCR cycling, and (c) using b-actin as the control where line 1 refers to exposed fish and line 2 to the control.

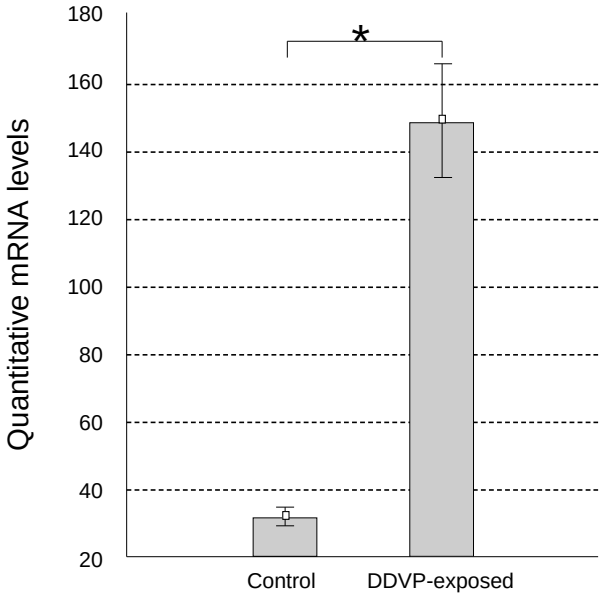

Fig. 2. Mean Hsp70 levels in quantitative mRNA differed between DDVP-exposed fish and control at $p<0.01$.

Discussion

The present work demonstrates the adverse effect of DDVP on Hsp70 gene expression. While the quantitative mRNA level was very low in the control after forty PCR cycles, it was very high in DDVP-exposed fish. Many experiments deal with the adverse effects of DDVP on organisms. Shih and McDonough (1997) suggested that the primary effect of dichlorvos and other organophosphorus compounds on vertebrate and invertebrate organisms is the inhibition of the enzyme acetylcholinesterase (AChE) which is responsible for terminating the transmission of nerve impulses. McHenery et al. (1997) showed that experimental exposure of mussels (*Mytilus edulis* L.) to dichlorvos resulted in changes in gill acetylcholinesterase activity with an increase at lower concentrations and 50% inhibition at 3.6 pg per liter after a 24-h exposure.

As a chemotherapeutant, DDVP has a greater toxic effect on crustaceans than on other aquatic invertebrates (Egidius and Moster, 1987; McHenery et al., 1990; Thain et al., 1990) and McHenery et al. (1991) showed that levels of organophosphorus compounds of 5.7 and 122 µg per liter affect crustaceans.

Ozen and Korkmaz (2004), Egidius and Moster (1987), McHenery et al. (1990, 1991), and Thain et al. (1990) found that organophosphorus compounds have an adverse effect on organisms but their effect on gene expression remains unclear. In our experiment, the organophosphorus compound DDVP adversely affected gene expression in trout. Therefore, it is possible that they also alter gene sequence.

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