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Detection of Cytochrome P450 1A Content Exposed to Polycyclic Aromatic Hydrocarbons (PAHs) in Two Species of Sturgeon

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Abstract: The cytochrome P450 1A subfamily is responsive to environmental contaminants such as polycyclic aromatic hydrocarbons. Because of its inducibility, it has used as an important tool for impact assessment of pollutants in aquatic environments. The enzyme linked immunosorbant assay (ELISA) for measuring cytochrome P450 1A in two sturgeons, Beluga, *Huso huso* and Persian Sturgeon, *Acipenser persicus* are described. Fish, Sturgeons (*Acipenser persicus* and *Huso huso*) were treated by single i.p. injection of $ –naphthoflavon (BNF) and 3- methylcolantheren (3MC) dissolved in corn oil at three doses 35, 70 and 105 mg/kg body weight/day respectively for 72 hours. Similarly control fish received an injection of only corn oil. Microsomal fraction was prepared and enzyme activity was measured with deethylation of ehtoxyresorufin reaction (EROD) by flourometery method. Immunodetetion of P4501A content was measured using ELISA technique. An indirect ELISA based on polyclonal rabbit anti-beluga cytochrome P450 1A IgG and monoclonal anti-cod P4501A1has been developed. ß-naphthoflovon treated-fish caused a 15-26 and 20-25 fold increase respectively in 7-ethoxyresorufin deethylase activity of *Huso huso* and *Acipenser persicus* liver microsomes than to controls. The results of ELISA were showed strong cross-reactivity of treated fish liver of microsomes as comparable to fish liver microsomal control. The strong cross reactivity was obtained in ELISA technique when the specific antibodies were applied. Polycyclic Aromatic Hydrocarbons (PAHs) could be induced cytochrome P450 1A gene and is increased the enzyme biosynthysis, which is raised the cytochrome P4501A content in two Sturgeon with ELISA assay.

Key words: Cytochrome P450 1A % *Huso huso* % *Acipenser Persicus* % ELISA

INTRODUCTION

As the results of high technological and industrial developments, the aquatic environment is becoming increasingly threatened by a number of organic compounds from industries, mining, agriculture and urban waste. Chemical analysis is sensitive but can expensive and provide little information on the potential effects of contaminants in aquatic environment [1]. So today, biological assays can be used to indicate the presence and potential impacts of toxic chemicals in aquatic animals. Organic pollution such as polycyclic aromatic hydrocarbons (PAHs) induces cytochrome P450 1A (CYP 4501A) isozyme in fish. The cytochrome P450 1A is paying an important role in biotransformation of xenobiotic. So, it has used as biomarker for aquatic organic pollution biomonitoring.

In mammals, the cytochrome P450 –dependent monooxygenase system has been shown to be responsible for the majority of cellular xenobiotic biotransformation [2-4]. Among many compounds metabolized by the cytochrome P450s are endogenous substances, such as fatty acid, steroids and prostaglandins, pesticides and carcinogens [5]. Today, over than 200 different cytochromes P450 1A capable of catalyzing a variety of potential substrates

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have been identified and cloned. The majority of these cytochromes P450 1A are selectively induced by specific group of compounds that differ from species to species [4].

The multiplicity of CYP 4501A and their selective induction have also been shown in marine and fresh water fish [6]. Search for biological indicators for environmental pollution motivated some of the earlier studies on CYP induction in fish. Exposure to petroleum products, polychlorinated biphenyls, dioxins and PAH has been reported to induce CYP in fish [4, 7]. It has also been shown that biotransformation of xenobiotic in fish occurs by many of the same enzyme systems and reactions that are found in mammalian species. Measurement of these activities has been suggested as possible means of monitoring environmental pollution [6, 8].

Enzymed-linked immunosorbent assay (ELISA) is an efficient tool for evaluating the level of a specific protein in protein mixture and many protocols have been developed to adapt the basic ELISA concept to different proteins and applications [9]. CYP 4501A ELISA assay was used for screening of potential anti -P450 antibodies or for determining P450 levels. Indirect ELISA method of choice for this application and its establishment should include purification of cytochrome P450 1A.

The purpose of this study is to investigate the effect of PAHs per mutagen compounds, beta-naphthoflavon and 3-methylcholantheren on liver microsomal activities and cytochrome P4501A content of Sturgeons using EROD assay and to determine the amount of Induced cytochrome P450 1A content by an indirect ELISA based on polyclonal antibodies against beluga cytochrome P450 1A.

MATERIALS AND METHODS

Chemicals: $-naphthoflavon, 3-methylcholanterem, bovin serum albumin (BSA),7-ethoxyresorufin, resorufin, nicotinamide adenine dinucleotide (NADPH) were purchased from Sigma Chemical Company. Horseradish peroxidase-conjugated anti-rabbit IgG, Tween 20, sodium dodecyl sulfate (SDS) were obtained Bio-Rad. Monclonal cod P4501A was prepared from Biosence Company. All the other chemicals were of analytical grade and were obtained from commercial sources at highest grade of purity available.

Fish: Juveniles of *H. huso* and *A. persicus* in the weight range of 252.40±3.82 g were obtained from the Shahid Beheshti proliferation and culture centre for sturgeon fish, Rasht, Iran. Fish were transferred to laboratory and acclimated for 2 weeks. Fish were received i.p. injection of BNF and 3-MCabout 35mg/ml/day fish body weight in corn oil respectively at three different doses. Control fish received the corn oil only.

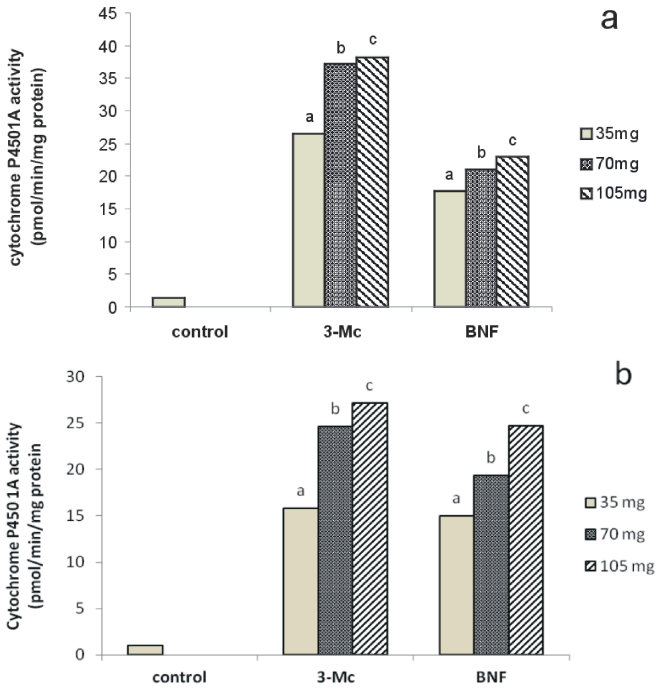
Prep Ration of Fish Liver Microsomes: The fish were killed by decapitation. The livers were removed and were placed in liquid nitrogen and transported until experiment.

Microsomes were prepared from fish liver by differential centrifugation [10]. The microsomes were washed by suspending them in 1.15% KCl containing 1mM EDTA and collected by centrifugation and the pellet was resuspended in 10% glycerol containing 1Mm EDTA and stored in liquid nitrogen until use. Protein concentration was determined by the method of Lowry *et al*. [11] using bovine serum albumin as a standard. The enzyme activity was measured with the formation of fluorescent metabolite, resorufin in 7-ehoxy resorufin-O-deethylase reaction by the fluorimtric method [12]. Microsomes were diluted to a concentration about 10 µg /ml in 50 mM bicarbonate buffer with pH 9.5 and adsorbed to microtiter wells for two hours at 37°C at 100 µl per well. After washing the wells, the wells were blocked for unspecific binding with 200µl 5% Tween 20 in Tris -buffer (TBS) for 20 minutes at room temperature. Primary antibody (rabbit anti- sturgeon P450 1A IgG) was diluted 1:10000 in TBS added to the wells (100µl) and incubated for two hours at 37°C. After washing four times with Tris–buffer with tween (TBST), 100µl of secondary antibody (GAR-HRP) at a 1:6000 dilution in TBS added to each well and incubated for one hour at 37°C. The last washing was repeated six times and then 100µl of TMB substrate was added to each well. The reaction was developed for 10-30 min and was stopped by addition of H_2SO_4 to each well [9, 13, 14].

Statistical Analysis: Data were expressed as mean ± standard deviation and were analyzed by analysis of variance (ANOVA); using SPSS 10.0 software was employed to determine the comparisons. The significance of results was ascertained at p-value less than 0.05.

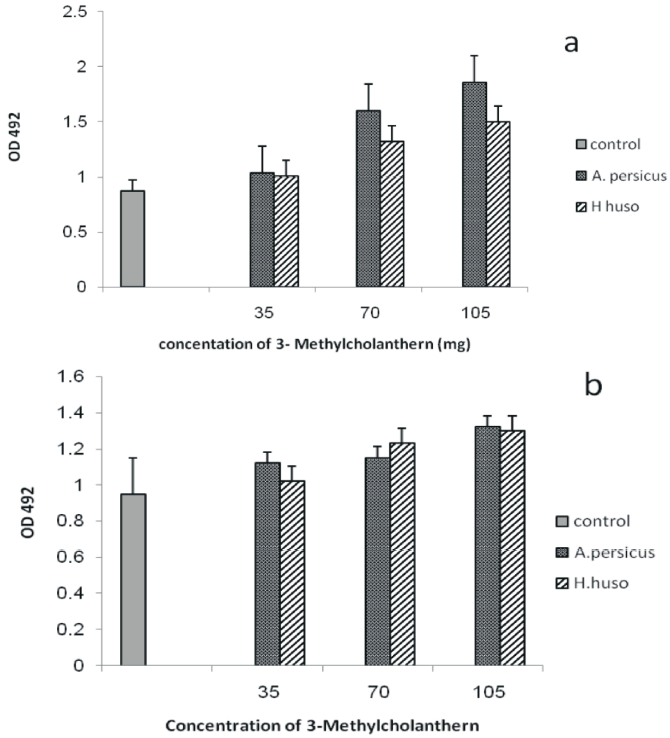
RESULTS

The results of ß-naphthoflavon and 3-methylcholantheren induction were shown in Figure 1. The treated- *H. huso* and *A. persicus* caused a 20-25-fold increase respectively in 7-ethoxyresorufin –O-deethylase (7-EROD) activity of liver microsomes than the control fish.



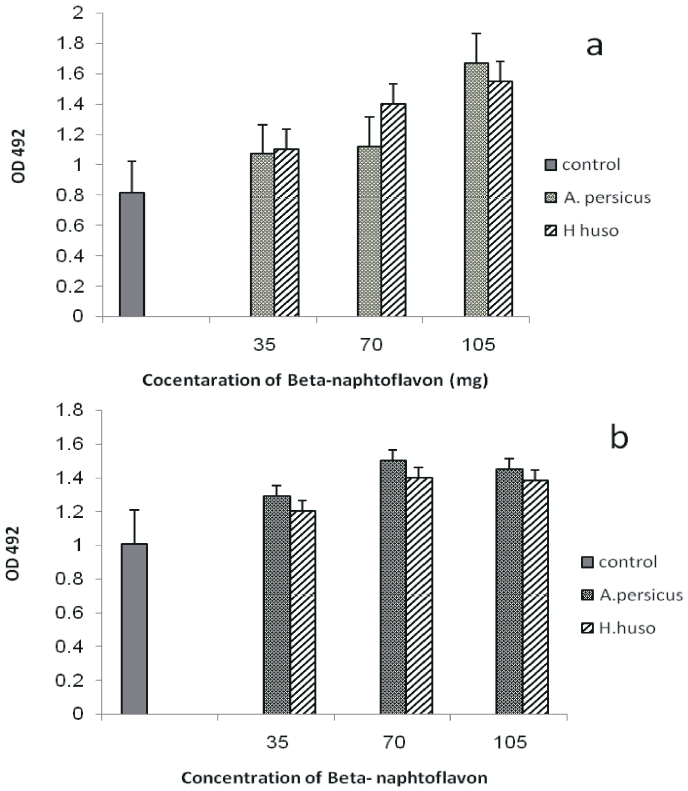
The results are mean ±SDE; n=6. Significantly different from Control.

Fig. 1: Effect of different concentration of 3-Methylcholantheren and Beta-naphtoflavon on cytochrome P450 1A activity (EROD activity) in a) *Acipencer persicus* and b) *Huso huso*.



The results are mean ±SDE; n=6. Significantly different from Control.

Fig. 2: Effect of different concentration of 3-Methylcholantheren on cytochrome P4501A content in *A. persicus* and *H. huso* a) monoclonal Cod P4501A and b) polyclonal anti-beluga P450 1A.



The results are mean ±SDE; n=6. Significantly different from Control.

Fig. 3: Effect of different concentration of Beta-naphtoflavon on cytochrome P4501A content in *A. persicus* and *H. huso* a) monoclonal Cod P4501A and b) polyclonal anti-beluga P450 1A.

Immunodetction of treated liver microsomes of beluga and Acipenser with ELISA assay were observed in Figures 2 and 3. The results of using different antibodies in cytochrome ELISA assay were shown strong cross-reactivity with the polyclonal anti-beluga P450 1A IgG prepared in our Lab. and cross reactions were compared with of the monoclonal P4501A1 preparation.

DISCUSSION

Several studies have shown that fish respond to 3-methylcholanthern and ß-naphthoflavon compounds induction in cytochrome P450 1A synthesis [2, 10, 12, 15, 17]. In this study, treatment of *Huso huso and Acipenser persicus* with these compounds has resulted in induction of liver EROD activity, which is associated with cytochrome P450 1A.

Immunological studies using polyclonal anti- beluga P450 1A used in the ELISA technique was tested for cross –reactivity with the other fish species and rat. The results demonstrated that anti-beluga (*Huso huso*) P4501A IgG cross- reacted with single protein band in the 54-59 kDa region in the liver microsomes of fish. This protein band was induced by 3-methylcholantheren and ß-naphthoflavon treated fish [18]. These results, were showed that the immunogen was the major P450 form purified from BNF treated fish liver microsomes.

Formation of new cytochrome P450 1A with an apparent molecular weight of about 58kDa on electrophoresis pattern and significant induction of EROD suggested that PAHs induce cytochrome P450 1A in Sturgeon liver microsomes. Also, we found an excellent agreement between cytochromeP4501A content using ELISA assay and EROD activity with an overall correlation of about 0.84. So the ELISA technique is simple and rapid screening of a large number of samples simultaneously in comparable to enzyme activity (EROD) and it could be used as tool for biomonitoring program.

The results of this study demonstrate that polycyclic aromatic hydrocarbons such as ß-naphthoflovon and 3-methylcholanthern has the ability to induce P450 1A activity. Induction mechanism involves elevation of microsomal protein expression from fish ctyochrome P450 1A which is indicated in the cytochrome P450 1A content using ELISA assay.

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