BIOMONITORING OF TOXIC , CARCINOGENIC POLLUTANTS BY MOLECULAR AND BIOCHEMICAL RESPONSES OF FISH CYTOCHROME P4501A1 (CYP1A1) ALONG THE IZMIR BAY ON THE MEDITERRANEAN SEA

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Abstract

In this report we present biomonitoring of toxic carcinogenic organic compounds by using induction of CYP1A1 mRNA, protein and 7-ethoxyresorufin O-deethylase (EROD) activity as biomarkers in liver of leaping mullet (*Lisa saliens*) caught along the Izmir Bay. Pasaport containing the highest concentrations of petroleum hydrocarbons (12.45μg/L) and the other sites (sites 2,3,4,and 11) showed significantly increased EROD activity and CYP1A1 protein level as compared to the values from uncontaminated (<1μg/L) reference site, Foca. These conclusions were further confirmed with the results of fish liver CYP1A1 mRNA level measurements.

Key words: Pollution, Biomonitoring, Enzymes, CYP1A1, PCB, PAHs, Carcinogens.

Introduction

With the recent advances in molecular biology and biochemistry, responses of fish and other organisms to pollutants are used for the ecological impact assessment of these compounds in the aquatic environment to detect both the early and long term effects and to examine also their possible genotoxicity. The best characterized and used biochemical marker so far is the induction of CYP1A in fish liver (1,2). Organic pollutants such as dioxins, dibenzofurans, polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCBs), specifically induce liver CYP1A in fish and in other vertebrates. CYP1A mostly converts certain classes of PAH procarcinogens and other organic chemicals to their carcinogenic metabolites by forming epoxides or other oxygenated compounds. Thus, induction of CYP1A is used to measure both exposure and resulting toxic, carcinogenic effects of these types of organic pollutants.

Izmir Bay is located on the Aegean Sea of the Mediterranean, west of Turkey. Domestic and industrial wastes, urban, agricultural run off, discharges from ships, sediments and contaminated waters of rivers heavily pollute the Bay (3). Although, the contamination of Bay with the heavy metals has been monitored since 1960s, both the degree and specific sites of pollution by the organic carcinogenic compounds and the biological effects of monitoring in the Bay have remained unexplored until recently (4).

We have carried out biomonitoring studies by using CYP1A1 dependent enzyme activity induction in leaping mullet (*Lisa saliens*) and in common sole (*Selea vulgaris*) liver measuring 7-ethoxyresorufin O-deethylase (EROD) activity, CYP1A1 protein amount by western blotting on the several sites in Izmir Bay since 1995. In the most recent studies, the degree of the increased liver CYP1A1 mRNA level of leaping mullet was added to our monitoring studies. In this communication, biomonitoring of toxic carcinogenic organic compounds by using these three parameters, that is, induction of CYP1A1 mRNA, protein and EROD activity in fish liver will be presented. Sequence analysis of cDNA cloned from liver of leaping mullet collected from site 1, Pasaport demonstrated that it is CYP1A1 (Sen *et al.*, unpublished. GenBank Accession Number AF072899).

Sampling sites and fish :

The collection sites, all were in Izmir Bay, are given in Fig.1. Leaping mullets, each weighing 300-400 g, were caught by fish net.

Methods

Preparation of microsomes, determination of protein and EROD activity were described elsewhere in detail(4). Cytochrome P4501A1 protein levels were determined by Western blot analysis using polyclonal antibodies (anti-mullet CYP1A1) produced in our laboratory against purified leaping mullet CYP1A1 as described before (4).

Total cellular RNA was isolated from the liver tissue of leaping mullets collected from different stations essentially as described before (6). Following the electrophoresis and transfer of RNA samples, Northern blot hybridization was accomplished by using the synthetic new 33-mer oligonucleotide probe designed for this purpose, 5'-dCTC ATC CAG CTT CCT GCT CTC GCA GTG ATC AAT-3'. Details of the procedure are given elsewhere in detail (6).The results are subjected to Student's t-test for the statistical analysis.

Results and Discussion

The results of .biomonitoring studies carried out in January 1999 are given in Fig. 1. EROD activities of leaping mullet from site 1 (Pasaport) in the inner Bay showed highly elevated enzyme activities (1028 ± 287 pmol/min/mg, n=4) which were 93 times higher than the value at reference site 2 (site 8 on the Fig. 1). Leaping mullets caught from site site 6 and site 11 showed moderately elevated EROD activities that were 105± 26 (n=4) and 310± 21 (n=4) pmol/min/mg protein, respectively. In our first monitoring studies carried out in 1995 and 1996 using leaping mullet liver CYP1A1 enzyme activity, EROD, similar results were obtained

The leaping mullet from the highly urbanized and industrial section of the Bay, Pasaport (site1) showed highly elevated enzyme activities (1293± 292 pmol/min/mg protein, n=208), which were about 62 times higher than the value at the reference site 1, (site 10 in Fig. 1). (25 ± 9 pmol/min/mg protein, n=4).Leaping mullet caught along a pollutant gradient at three other sites, Karsiyaka (site 2), Inciralti (site 3), and Tuzla (site 4), also had highly elevated EROD activities, as can be seen in Fig. 1 (4).

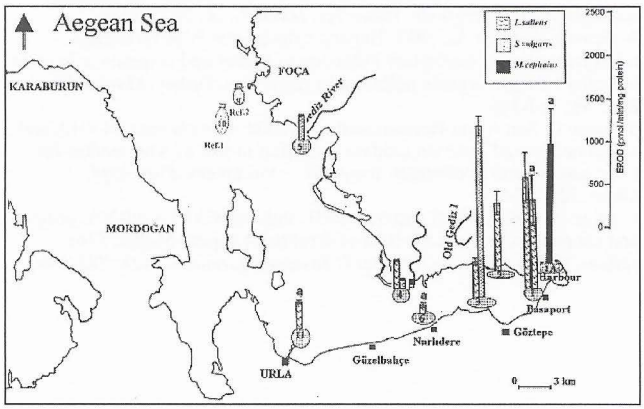

Figure 1. EROD activities of liver microsomes of common sole (*Solea vulgaris*), leaping mullet (*Liza saliens*) and gray mullet (*Mugil caphalus*) captured in Izmir Bay at ten different sampling sites.
The activity bars with "a" represent the field studies carried out in 1999. The others are carried out in 1995 and 1996. Compiled from the references 4, 5 and 6.

In both biomonitoring studies (1996 *vs.* 1999), leaping mullet sampled from site1, Pasaport containing the highest concentrations of petroleum hydrocarbons (12.45 μg/L) showed the highest liver EROD activity. In addition, these livers had highest CYP1A1 protein levels as determined by Western blotting. Next to site1, mullet captured from site 2 and site 3 also had very high EROD activities and a good correlation was obtained between the EROD activity and CYP1A1 protein content measured immunochemically as shown in Fig. 2A.

Thus, these molecular and biochemical indices, highly induced EROD activities, CYP1A1mRNA and protein levels in the liver of leaping mullet caught from sites 1,2,3 and 4 suggests that these sites are highly contaminated with carcinogenic PAHs and / or PCBs and possibly other toxic compounds.

The results obtained from CYP1A1 protein and EROD activity were further supported by CYP1A1 mRNA studies of leaping mullet. As seen in Fig. 2B, RNA isolated from the liver of mullet collected from the most polluted site revealed considerably strong hybridization signal whereas only a weak hybridization signal was detected with liver RNA of fish caught from reference point, site10

This results also suggest that CYP1A1 gene expression is transcriptionally induced by chemical pollutants in the contaminated sites. Thus, these molecular and biochemical indices, i.e., highly induced EROD activities, CYP1A1mRNA and protein levels in the liver of leaping

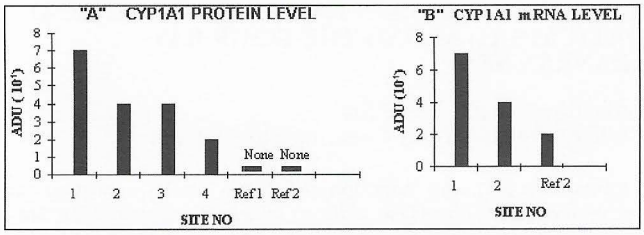

Figure 2. CYP1A1 protein ("A") and CYP1A1 mRNA ("B") levels in liver microsomes of leaping mullet caught from the several sites in Izmir Bay. *Numbers below the bars correspond to the collection sites given in the map in Figure 1. The bar graph "A" illustrates arbitrary densitometric units (ADU) obtained from Western blots of liver microsomes. Quantitation of CYP1A1 mRNA bands from Northern blots were obtained by using scanning densitometer and also expressed as ADU and given in graph "B". Ref1 and Ref2 are sampling sites 10 and 8, respectively., in Fig.1.*

mullet caught from sites 1,2,3,4 and 11 suggests that these sites are highly contaminated with carcinogenic PAHs and/or PCBs and possibly other toxic compounds. These sites are prone to the alarming pathological changes at the population and community levels.

References

1- Payne J.F. *et al.*, 1987. Review and perspective on the use of mixed function oxygenase enzymes in biological monitoring. *Comp. Biochem. Physiol.*, 86C: 233-245.

2- Buhler D.R. and Wang-Buhler J.L.,1998. Rainbow trout Cytochrome P450s: purification, induction, and role in environmental monitoring. *Comp. Biochem. Physiol.*, 121C: 107-137.

3- Balkas I.T. *et al*,1992. Izmir Bay wastewater management project-economical considerations. *Water Sci. Technol.*, 26: 2613-2616.

4- Arinc E and Sen A., 1999. Hepatic cytochrome P4501A1 and 7-ethoxyresorufin o- deethylase induction in mullet and common sole as an indicator of toxic organic pollution in Izmir Bay, Turkey. *Marine Env. Res.*, 48: 147-160.

5- Arinc E, Sen A and Bozcaarmutlu A., 2000. Cytochrome P4501A and associated mixed function oxidase induction in fish as a biomarker for toxic carcinogenic pollutants in aquatic environment. *Pure Appl. Chem.*,72: 985-994

6-Arinc E , Kocabiyik S and Su . 2001. Induced CYP1A mRNA, protein and catalytic activity in the liver of feral fish Leaping Mullet, *Lisa saliens*, *Comp. Biochem. Physiol C Toxicol Pharmacol.*, 128: 281-290.

Introduction

Au cours de ce travail, nous avons pu voir que la survie de *P. aeruginosa* est assez longue comparée à celle des eubactéries. L'adaptation de ce germe aux conditions marines se fait par modification des caractères morphologiques (réduction de la taille, diversification de la formes des bactéries) et modification des caractères culturaux : apparition des colonies rugueuses ou lisses à pigment jaune localisé au niveau des colonies ainsi que des colonies oranges et noires.

Matériel et méthodes

Préparation inoculation et incubation de la souche étudiée :

La souche de référence de *Pseudomonas aeruginosa* ATCC 27853 est incubée pendant 6 à 12 ans dans l'eau de mer plus du sédiment marin. Elle est cultivée à 37 °C pendant 24 heures sur une gélose nutritive. Elle est par la suite récupérée dans de l'eau physiologique et inoculée dans un flacon Erlen meyer renfermant 200 ml d'eau de mer stérile.

Milieux de culture utilisés:

Gélose nutritive (GN), Bouillon nutritive (BN),Gélose nutritive préparée à l'eau de mer (GNEM), Mueller hinton (MH), King A préparée à l'eau de mer (KAEM). L'étude des caractères biochimiques est faite sur des galeries Api 20 NE.

Résultats

1) Modification des caractères culturaux de P . aeruginosa au cours de son incubation en eau de mer.

P. aeruginosa incubé six ans dans de l'eau de mer additionné de sédiment marin et incubé trois jours dans du BN donne :

- des colonies blanches avec centre jaunâtre sur GN qui gardent leur aspect sur KAEM, des grandes colonies jaunes et blanches et d'autres naines.
- des colonies de taille variable entre 1 et 2 mm de couleur blanche avec un anneau transparent au milieu qui, par alternance de repiquages sur BN, GN et MH donnent des colonies oranges, blanches et d'autres jaunes rugueuses. Ces dernières donnent à la suite de plusieurs repiquages sur GN et KAEM des colonies blanches rugueuses qui deviennent jaunes après 48 heures de leur apparition sur ce dernier milieu et des colonies blanches sur GNEM.

P. aeruginosa incubé dix ans dans de l'eau de mer additionnée de sédiment marin ne donne pas de colonies après incubation de trois jours dans du BN et 24 heures sur GN mais c'est après environ une quinzaine de jours que les colonies naines poussent. Incubé trois mois dans du BN *P. aeruginosa* donne des formes très diversifiées avec des pigments orange et jaune. Les colonies orangées, au début transparentes donnent par vieillissement sur GN (2mois) des colonies noires bombées, desséchées incrustées dans la gélose. Dans un milieu liquide, elles donnent des amas indissociables. Une fois ensemencées, ces dernières donnent par repiquage sur GN des colonies transparentes puis orangés de tailles plus ou moins grande. Ces mêmes colonies ne donnent pas les mêmes profils biochimiques sur Api 20NE mais elles présentes la même résistance aux antibiotiques.

2) Modification des caractères biochimiques :

L'étude des ces modifications sur des galeries Api 20 NE, nous a permis de voir que *P. aeruginosa* au cours de son incubation en eau