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Short Communication

Zebra Fish as a Model for Assessing Environmental Toxicology: Expression of Antioxidant Biomarker Genes

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Abstract

Zebra fish is preferred as a model organism because of the ease with which zebrafish development is followed from the transparent egg stage onwards; it is possible to scrutinize the defects in different developmental stages by xenobiotic onslaughts. It has been amply recorded that most of the xenobiotics go through the three phases of detoxification covering hydroxylation, reduction and oxidation in Phase I, conjugation in Phase II and excretion in Phase III. Xenobiotics trigger differential gene expression patterns of Phases I and II affecting the rate of transcription of the biotransformation enzymes. An active Nrf2/Keap1 system also imparts a global role of zebrafish in detoxification. Majority of the antioxidant genes are expressed by different types of xenobiotics both in embryo and adult stages of zebrafish. Thus zebrafish attains the status of the most reliable model in environmental toxicology to unravel the molecular mechanisms of detoxification.

INTRODUCTION

Zebra fish (*Danio rerio*) is a very reliable model for investigations on xenobiotic toxicity, drug discovery and human disease [1]. There are several advantages in using zebrafish as a toxicological model species among which the two main advantages are: (i) small size (approximately 1.5 inches long) that reduces housing space, husbandry costs as well as breeding and maintenance cost and (ii) assessment for toxicological endpoints. [1]. Zebrafish development can be visualized right from the egg stage. The optical clarity of the eggs allows easy developmental staging, identification of phenotypic traits during mutagenesis screening and assessment of toxicity endpoints during toxicity testing [1]. Zebrafish offers the unique combination of transparent and accessible embryos, cost-effective mutagenesis screening, full sequenced genome and knock out technology [2].

Zebra fish embryo may be used as a robust biological sensor in high-throughput characterization of multidimensional *in vivo* effect of hazardous chemicals. A global pattern of variation in the response of zebrafish embryos to 1060 U.S. EPA ToxCast Phase I and II compounds utilizing 18 simultaneously measured endpoints is also on record. It was observed that 487 out of 1060 compounds induced significant biological responses in tested embryos [3].

A biomarker, or biological marker, generally refers to a measurable indicator of some biological state or condition and an environmental agent that can be chemical, physical or biological in nature. The term biomarker may be divided into three types- (i) biomarker of exposure, (ii) biomarker of response or toxic effect and (iii) biomarker of susceptibility [4].

Xenobiotics and/or endogenous toxic metabolites are harmful to cells and the inherent defense mechanism triggers the cellular machinery to induce the Xenobiotic Metabolizing Enzymes (XMEs). Liver actively takes part in detoxification that mainly occurs through Phase I-III detoxification systems. The purpose of these systems is to increase the hydrophilicity of endogenous or exogenous substances (xenobiotics or pollutants) through hydrolysis, reduction, oxidation (Phase I) followed by conjugation (Phase II), and finally the efficiency of transport and excretion (Phase III) [5].

Xenobiotics are known to affect gene expression pattern of Phase-I and Phase-II detoxification via binding to the aryl hydrocarbon receptor (AHR). Upon ligand binding, these receptors translocate into nucleus and bind with Xenobiotic Response Element (XRE) or Antioxidant Response Element (ARE) that enhance the rate of transcription of the biotransformation enzymes [6]. The regulation and activity of specific Phase I or II metabolizing enzymes are strong indicators to assess

the effectiveness of xenobiotics such as benzo [a] pyrene and inorganic arsenic in zebrafish [7]. Cytochrome P450 (CYP), a Phase-I enzyme, has a primary role in the metabolism of halogenated compounds which has been used as a central biomarker for assessing environmental contamination in aquatic species, particularly in fish [8].

Cellular systems evolved several antioxidant proteins and Phase-II detoxifying enzymes to counter stress [9]. The transcription factor, familiar as nuclear factor (erythroid-derived) like 2 (Nrf2), is responsible for induction of Phase-II genes. Nrf2, a member of the "cap 'n' collar" basic region-leucine zipper (CNC bZIP) transcription factor, has been recognized as the master regulator of cellular defense mechanism against toxic insults [10]. It has been found that hypoxic condition causes enhancement of steady state levels of Nrf2 mRNA expression in null mice as compared to the wild mice [11]. Another protein which can interact with Nrf2 in the cytoplasm is Keap1 (Kelch-like ECH associating protein 1) which negatively regulates Nrf2. In the basal state, Nrf2 binds with Keap1 to form an Nrf2-Keap1 complex in the cytoplasm. But during stress or toxic insults Nrf2 dissociates from the complex and translocates to nucleus to bind with ARE that leads to the transactivation of cytoprotective genes like heme oxygenase 1(Ho-1), NADPH quinone oxygenase 1(Nqo-1) and glutathione-s-transferase (Gst). Nrf2/Keap1 system is supposedly present in all vertebrates including zebrafish indicating its global role on cellular detoxification mechanism [12-15].

Experimental analysis on zebrafish Nrf2 gene knock-down using morpholino-phosphorodiamidate-modified antisense oligonucleotide clearly demonstrated that Nrf2 plays an important role in Phase-II gene induction in fish. Keap1 also exists in zebrafish which interacts with Nrf2 as a negative regulator. However, the molecular mechanism of Nrf2 regulation in fish has not received due attention [16,17]. Expression of downstream genes in the Nrf2 regulated antioxidant pathway [Catalase (Cat), copper/zinc superoxide dismutase (Cu/Zn Sod), manganese superoxide dismutase (Mn Sod), glutathione peroxidase (Gpx1), Gst, cytochrome c oxidase1 (Cox1), Uncoupling protein2 (Ucp2) and B-cell lymphoma 2 (Bcl2)] are therefore crucial to understand the detoxification mechanism of xenobiotics at a molecular level.

The expression of antioxidant genes in zebrafish embryo after individual and combined exposure of aryl hydrocarbon receptor agonist b-naphthoflavone (BNF) and cytochrome P4501A inhibitor a-naphthoflavone (ANF) was examined; knockdown of Nrf2 increased mortality following tBOOH challenge, prevented significant up regulation of antioxidant genes following both tBOOH and BNF + ANF exposures, and exacerbated BNF + ANF–related deformities [18]. The findings demonstrated that antioxidant responses are a component of polycyclic aromatic hydrocarbon (PAH) synergistic developmental toxicity and that Nrf2 is protective against pro-oxidant and PAH challenges during development. It was further demonstrated that oxidative stress and expression of immune related genes in zebrafish embryos subjected to short–term exposures to varying concentrations of di-n-butyl phthalate (DBP), di-ethyl phthalate (DEP) and a mixture of DBP and DEP inducing antioxidant enzyme activities and also transcription of innate immune-related genes at early developmental stages (4 h and 96 h post fertilization) [19].

Zebra fish is a premier model for vertebrate development [20] and toxicology [1]. Liver is known to be a major target organ of arsenic toxicity in mice [21] and human [22]. DNA and protein damage resulted by arsenic induced oxidative stress can lead to differentially expressed genes encoding several proteins related to DNA damage /repair, antioxidant activity, hypoxia, heat shock response, arsenic metabolism, iron homeostasis and ubiquitin-dependent protein degradation [23]. These findings were corroborated in comprehensive transcriptomic analyses of arsenic exposure where among 1444 differentially expressed genes in zebra fish liver, 750 genes were up regulated and 694 down regulated [24]. More recently, it has been reported that in adult female zebrafish liver differential expression patterns of several antioxidant genes were noteworthy; Cyp1A mRNA increased in a dose dependent manner up to 30 mg NaF/L in 30 days of exposure groups but decreased in 15 mg NaF/L in 90 days treatment groups. The mRNA expression of 15 and 30 mg NaF/L for 30 days treatment groups were highly significant. This pattern was just reverse in the case of Ho1where only 15 mg NaF/L for 90 days exposure was highly significant. Nqo1 increased in all the treatment groups respectively. The mRNA levels of Mn Sod and Gpx increased in a dose dependent manner. However, the transcriptional expression of Cu/ZnSod and Gst increased in a time dependent manner both at 15 mg/L for 30 and 90 days exposure periods whereas Cat remained unaltered in both the treatment groups. Ucp2 gene synthesizing protein located in the mitochondrial inner membrane in response to ROS production, increased in both the treatment groups. Hsp70 mRNA level remained unchanged after 30 days and was down-regulated significantly after 90 days treatment [25]. Elevated expression of Nrf2 protein was found in liver of all the treatment groups. 15 mg NaF/L treatment had higher Nrf2 expression level than other groups whereas Keap1 decreased in all the treatment groups. This decrease was most prominent in 15 mg NaF/L treated fish for 90 days. However, these alterations in proteins were not dose dependent [13].

The expression patterns of antioxidant genes were studied in adult zebrafish brain both after arsenic [14] and fluoride [15] treatment. The transcriptional level of Cat, p38 and Nqo1 gene increased significantly in case of fluoride exposure. Cu/Zn Sod, Mn Sod, and Gpx mRNA level also increased though Ucp-2 mRNA level remained same after both arsenic and fluoride exposure. The mRNA level of Nrf2 was significantly high after fluoride treatment but a triphasic pattern was observed in case of arsenic. Fluoride treatment decreased Keap1 mRNA level which showed a biphasic pattern in case of arsenic exposure. Nrf2 protein expression increased in a dose dependent manner after fluoride exposure and towards the end of the arsenic exposure. There was a dose dependent decrease in Keap1 protein after fluoride exposure but arsenic treatment showed a biphasic pattern.

It is surmised that the expression of antioxidant biomarker genes in zebrafish can give a detail insight into the toxicity-detoxification mechanisms induced by different xenobiotics. Further studies in this direction are warranted to unravel the specific molecular pathways triggered in the progression of cellular detoxification. It is concluded that zebrafish is an extremely reliable aquatic model in researches on environmental toxicology to assess critical biological responses.

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