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cDNA Cloning and Expression of a Cytochrome P450 1A (*CYP1A*) from the Pale Chub, *Zacco platypus*

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The pale chub (*Zacco platypus*) is generally found in Asian countries, such as Korea, Japan, and China. Nevertheless, very little information exists about the genes involved in the metabolism of xenobiotics in this species. This species is useful in monitoring the environmental impact on various pollutants in freshwater as a sentinel fish species. We cloned the full-length cDNA sequence of xenobiotic metabolizing cytochrome P450 1A (*CYP1A*) gene from *Z. platypus* and characterized it. Tissue distribution and time-dependent induction of *CYP1A* were studied by real-time RT-PCR. Induction pattern of *CYP1A* was studied by exposing the fish to an arylhydrocarbon receptor agonist, β-naphthoflavone (BNF). The liver showed the highest level of expression in basal state as well as BNF- treated fish. However, appreciable levels of expression were also recorded in Gill and kidney and the least level of expression was observed in the eye. The results of the time-course study revealed an induction in the liver, brain, and gills after 6 h and 12 h in most of the tissues. This study provides an insight into the xenobiotics metabolizing system of *Z. platypus* and offers baseline information for further research related to biomarker, stress, and adaptive response of this ecologically important fish species in the freshwater environment.

Key words : *Zacco platypus, CYP1A*, β-naphthoflavone, gene expression, xenobiotic metabolism

INTRODUCTION

Expression of particular genes is often associated with a specific physiological function or response to environmental changes and is a good indicator for exposure to a variety of environmental factors (Rees *et al.*, 2005). Among them, *CYP* genes not only play an important role in the metabolism of xenobiotics, but they also play a significant role in steroid metabolism (Williams *et al.*, 1998; Hu *et al.*, 2004; Goldstone and Stegeman, 2006; Isin and Guengerich, 2007). *CYP1A* induction, or the increased expression and abundance of *CYP1A* mRNA, is a well-documented phenomenon in teleost fish (Billiard *et al.*, 2004; Jönsson *et al.*, 2004; Ortiz-Delgado and Sarasquete, 2004) and occurs when planar halogenated compounds (PAHs, PCBs, TCDD etc.) bind to a cytosolic ligand-activated transcription factor known as the Ah (aryl hydrocarbon) receptor (Hahn and Stegeman, 1994; Sarasquete and Segner, 2000).

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Although *CYP* genes are one of the most extensively studied genes in mammals as well as in fish, sequence and expression profile of *CYP* genes in Cypriniformes species are not well documented except for a few well-known species such as a zebrafish (Gao *et al.*, 2011) and a minnow (Liu *et al.*, 2008).

Pale chub (*Zacco platypus*, Cypriniformes) was a suitable sentinel species because of their relatively high abundance in most rivers and streams of Korea. Also, this species has high mobility (Yamazaki *et al.*, 1996), lives in riffles of midstream and downstream, prefers sand and gravel bottom conditions, and feeds mainly on insects, algae, detritus, and micro-crustaceans (Kim, 1997). During the spawning period they are widely scattered in the waters from 30 to 50 cm depth (Nakamura, 1952). However, most fishes migrate to the deeper and warmer water column in winter (Nakamura, 1952; Kim, 1997).

Although population dynamics and ecological studies in *Z. platypus* have been done, only a few studies have been performed on on metacercarial infection (Park *et al.*, 2004; Cho *et al.*, 2006), immunohistochemical studies (Ku *et al.*, 2004), stressor identification and health assessment (Yeom *et al.*, 2007), and a chromosomal toxicity assay (Hayashi *et al.*, 1998). In addition, to date no information is available on gene expression profiles, xenobiotic metabolism and detoxification. The habitat of *Z. platypus* may be contaminated by environmental pollutants and it also feeds on insects which might have been contaminated and bioaccumulated insecticides. Therefore, information of xenobiotic metabolizing genes is desirable to understand responses of *Z. platypus* to exposure to xenobiotics directly or indirectly. As stated above, *Z. platypus* is a hardy fish and survives well under laboratory conditions. This species can be used as an alternate model for short and long term toxicity testing. Therefore, detailed molecular biological information on various genes from *Z. platypus*, especially those involved in toxicant metabolism (*CYP1A*, most notably), is desirable. In addition, there are a number of other QRT-PCR assays that have been developed to measure *CYP1A* mRNA expression in fish (Campbell and Devlin, 1996; Miller *et al.*, 1999; Cousinou *et al.*, 2000) that provide good models for data comparison. Here, we describe the full-length cDNA sequence of *Z. platypus CYP1A*, its tissue specific expression, and induction by a universal inducer of *CYP1A* and a potent agonist of the aryl hydrocarbon receptor, β-naphthoflavone to provide baseline information for further research related to biomarkers, as well as stress and adaptive response of this ecologically important fish species.

MATERIALS AND METHODS

1. Fish

Z. platypus (body length, 5.5 ± 0.3 cm; body mass, 1.7 ± 0.4 g) were caught in the Cho-Jeong stream (Ga-phyeong, Kyoung-gi, Korea) by using a gill net. Fish were acclimated to laboratory conditions for two weeks at 19°C and a photoperiod of 12 h light and 12 h dark cycles in a 100 L tank containing well-aerated water (pH 7.3, dissolved oxygen, DO 6.9 mg L^{-1}). During the acclimation period, fish were fed a commercial diet.

2. RNA isolation, reverse transcription, first strand cDNA synthesis

Seven tissues (brain, gill, intestine, liver, kidney, muscle and skin) were quickly dissected under sterile conditions and homogenized in three volumes of Trizol® reagent (Invitrogen, Paisley, Scotland) with a homogenizer. Total RNA from the fish was isolated using Trizol® reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturers' protocol. RNA qualities were confirmed spectrophotometrically (A260/A280 ratio≈1.8−2.0), respectively after treatment with a DNase at 37°C for 30 min to account for contamination of genomic DNA. Single-stranded cDNA was synthesized from 1 μg total RNA using an oligo $(dT)_{20}$ primer and PrimeScript™ 1st strand cDNA synthesis Kit (Takara, Japan) by reverse transcription according to the manufacturers' protocol.

3. PCR, amplification of 3′ and 5′ ends and sequence analysis

Degenerative primers were designed using conserved domains after multiple alignments of previously reported full-length cDNA sequences of teleosts *CYP1A*. The detail of primers and PCR conditions for amplification of partial sequences are given in Table 1. For amplifying partial sequences, RT-PCR was carried out using 2 μM of each primer and hepatic cDNA as a template using Taq polymerase (TaKaRa Bio Inc.). Oligonucleotide

Table 1. Primer and PCR condition details.

Gene name	Oligo	Sequences (5′→3′) position	Nucleotide	Remarks	PCR condition
ZP-*CYP1A*	RT-F	GCGCCCTGGAGGAACACATCAGC	479～501	cDNA amplification	95°C/2 min: 35cycle of 95°C/ 20s, 55°C/30s, 72°C/1 min: 72°C/5 min
	RT-R	GCAAGCGCCGTTGCGTGGGAG	1370～1390		
	3GSP1	GAGATCTTCCGTCATTCCTCCTTCC	1129～1153	3′-RACE	94°C/3 min: 35cycle of 94°C/40s, 60°C/40s, 72°C/1 min: 72°C/5 min
	5GSP1	CGTGCTGGGAAGGATGCGCAGG	898～922	5′-RACE	
	5GSP2	GCATATCCCGCAGATCACGTTGGC	592～615		
	5GSP-walking	GGGAATCTTGGTGCGCATGA	98～117		
	qRT-F	CACATCAGCAAAGAGGGACTGTA	493～515	real-time RT-PCR	95°C/10 min: 40cycle of 95°C/15s, 55°C/15s, 72°C/33s
	qRT-R	TCCCGTCGGCGTTCAT	542～555		
ZP-β-Actin	qRT-F	CATTGCTGACAGGATGCAGAA	432～452		
	qRT-R	GGGCGATGATCTTGATTTTCA	482～502		

synthesis and sequencing was performed at Bioneer Co. (Daejeon, South Korea). The full-length sequence of *Z. platypus CYP1A* was deduced using a capfishing kit (Seegen, Carlsbad, CA, USA). The 3′ Rapid Amplification of cDNA Ends (3′ RACE) and 5′ RACE were performed using the primers and PCR conditions as detained in Table 1, following the manufacturer instructions.

4. Tissue distribution

Real-time reverse transcription PCR (RT-PCR) was performed to study tissue distribution pattern of the *CYP1A* gene. The gene distribution pattern was studied in the following tissues: brain, gill, intestine, liver, kidney, muscle and skin using oligo $(dT)_{20}$ primer and SuperScript™ III reverse transcriptase (Invitrogen) according to the manufacturers' instructions. The PCR conditions are described in Table 1. The PCR products were separated on 1% TBE agarose gels containing ethidium bromide (EtBr) and visualized on a Fluor-STM Multimanager system (Bio-Rad).

5. BNF-induced induction of *CYP1A*

Fish (n=30) were exposed to BNF (Sigma-Aldrich Co., MO, USA) through tank water. BNF was dissolved in DMSO and added to the water to yield a concentration of 1 μM (20 ppm of DMSO). Control group (n=30) tank water was mixed with DMSO to 20 ppm to match the level in the exposed group. The concentration of BNF is based on Weber *et al.* (2002) and Jönsson *et al.* (2003). Fish were fasted for two days before the exposure and no food was supplied during the exposure. Fish (n=5) were randomly sampled at 0, 6, 12, 24, 48 and 96 h of exposure, respectively, anesthetized by immersion in buffered 100 mg L^{-1} tricaine methanesulfonate (MS-222; pH 7.0; Sigma) and sacrificed. Brain, gill, intestine, liver, kidney, muscle and skin were dissected out for study of expression of *CYP1A* mRNA using real-time RT-PCR.

6. Real time RT-PCR

Real time RT-PCR was performed to study tissue distribution and the expression pattern of *CYP1A* mRNA in *Z. platypus* exposed in BNF. QuantiTect® SYBR® Green PCR Kit (QIAGEN) was used to detect specific PCR products under the condition that are shown in Table 1. Amplification and detection of SYBR® Green were performed with the 7500 Real-Time PCR system (Applied Biosystems, Lincoln, CA, USA). The *Z. platypus* β-actin gene (GenBank accession no. JN648711) was used as a reference to normalize the expression levels between the samples after comparison test Real time RT-PCR data were obtained as threshold cycle (C_T) value and used to calculate ΔC_T values (ΔC_T is the C_T of the target gene subtracted from the C_T of the reference gene) of each sample. Fold change for the relative gene expression to the control was determined by the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). All experiments were done in triplicate.

7. Statistical analysis

For the data analysis, one-way ANOVA followed

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 -9                                                                GGGGGATAG
  1 ATGGCTCTGACAGTTCTTCCCATTTTGGGTCCGATCTCTGTGTCTGAGAGCCTGGTGGCC
    M  A  L  T  V  L  P  I  L  G  P  I  S  V  S  E  S  L  V  A
 61 ATCATCACCATATGTGTGGTGTATCTGCTCATGCGCCTCATGCGCACCAAGATTCCCGAG
    I  I  T  I  C  V  V  Y  L  L  M  R  L  M  R  T  K  I  P  E
121 GGGCTCCAGAAGCTGCCGGGGCCGAAGCCTCTTCCCATCATCGGGAATGTGCTGGAGGTG
    G  L  Q  K  L  P  G  P  K  P  L  P  I  I  G  N  V  L  E  V
181 GGAAACAACCCTCACCTGAGCCTGACCGCCATGAGTCAGTGCTACGGCCCCGTCTTCCAG
    G  N  N  P  H  L  S  L  T  A  M  S  Q  C  Y  G  P  V  F  Q
241 ATCCAGATCGGCACGCGTCCGGTGGTGGTGCTCGGCGGGAACGACGTGATCCGGCAGGCT
    I  Q  I  G  T  R  P  V  V  V  L  G  G  N  D  V  I  R  Q  A
301 CTGATCAAACAGGGCGAGGAGTTCTCCGGACGTCCGGATCTGTACAGCACCAGGTTCATC
    L  I  K  Q  G  E  E  F  S  G  R  P  D  L  Y  S  T  R  F  I
361 AGCGACGGCAAGAGTCTGGCCTTCAGCACGGATCAGGTGGGAGTGTGGCGCGCGGCGG
    S  D  G  K  S  L  A  F  S  T  D  Q  V  G  V  W  R  A  R  R
421 AAGCTGGCCCTGAGCGCCCTGCGGACCTTCTCCACGGTGCAGAGCTCCGAGTACTCCTGC
    K  L  A  L  S  A  L  R  T  F  S  T  V  Q  S  S  E  Y  S  C
481 GCTCTCGAGGAGCACATCAGCAAAGAGGGACTGTACCTGATCGAGCGCCTTCACACCGTC
    A  L  E  E  H  I  S  K  E  G  L  Y  L  I  E  R  L  H  T  V
541 ATGAACGCCGACGGGAGCTTCGACCCTTTCCGGCACATCGTGGTGTCCGTGGCCAACGTG
    M  N  A  D  G  S  F  D  P  F  R  H  I  V  V  S  V  A  N  V
601 ATCTGCGGGATATGCTTCGGCCGCCGCTACAGCCACGACGACGACGAGCTGGTGGGTTTG
    I  C  G  I  C  F  G  R  R  Y  S  H  D  D  D  E  L  V  G  L
661 GTCAACCTGAGCGACGAGTTCGGGAAGATCGTGGGAAGCGGGAACCCTTCGGATTTCATC
    V  N  L  S  D  E  F  G  K  I  V  G  S  G  N  P  S  D  F  I
721 CCTATCCTGCGCATCCTTCCCAGCACGACGATGAAGAAGTTCATGGCCATCAACGCTCGC
    P  I  L  R  I  L  P  S  T  T  M  K  K  F  M  A  I  N  A  R
781 TTCAGCAATTTAATGCAGAAGATCGTCAAGGAACATTACGACACCTTCGACAAGAACAAC
    F  S  N  L  M  Q  K  I  V  K  E  H  Y  D  T  F  D  K  N  N
841 ATCCGTGACATCACCGATTCGCTCATCGAACACTGCGAAGACCGCAAGCTGGACGAGAAC
    I  R  D  I  T  D  S  L  I  E  H  C  E  D  R  K  L  D  E  N
901 TCAAACGTCCAAGTGTCCGACGAGAAGATCGTGGGAATCGTCAATGACCTCTTCGGAGCG
    S  N  V  Q  V  S  D  E  K  I  V  G  I  V  N  D  L  F  G  A
961 GGTTTCGACACCATCAGCACGGCTCTGTCCTGGGCGGTGGTGTATCTGGTGTCCTACCCT
    G  F  D  T  I  S  T  A  L  S  W  A  V  V  Y  L  V  S  Y  P
1021 GAGATCCAGGAGCGACTGCAGAGAGAGCTGAATGAAAAGGTCGGAATGGATCGTACGCCG
     E  I  Q  E  R  L  Q  R  E  L  N  E  K  V  G  M  D  R  T  P
1081 CGCCTGTCCGACAGAACGGAGCTGCCGTTCCTTGAGGCCTTCATCCTGGAGATCTTCCGT
     R  L  S  D  R  T  E  L  P  F  L  E  A  F  I  L  E  I  F  R
1141 CATTCCTCCTTCCTCCCGTTCACCATTCCTCACTGTACGTCTAAAGACACGTCGCTCAAC
     H  S  S  F  L  P  F  T  I  P  H  C  T  S  K  D  T  S  L  N
1201 GGATATTTCATTCCCAAAGACACCTGTGTGTTCATCAACCAGTGGCAGGTCAACCATGAC
     G  Y  F  I  P  K  D  T  C  V  F  I  N  Q  W  Q  V  N  H  D
1261 CCGGAACTGTGGAAAGATCCGTCGAGCTTCAACCCGGACCGCTTCCTCACGGCGGACGGT
     P  E  L  W  K  D  P  S  S  F  N  P  D  R  F  L  T  A  D  G
1321 ACGGACCTCAACAAGACGGAAGGAGAGAAGGTGCTGGTGTTCGGCCTGGGCAAGCGGCGC
     T  D  L  N  K  T  E  G  E  K  V  L  V  F  G  L  G  K  R  R
1381 TGCATCGGAGAGTCCATCGGACGAGCCGAGGTCTTCCTGTTCCTGGCCATCCTGCTCCAG
     C  I  G  E  S  I  G  R  A  E  V  F  L  F  L  A  I  L  L  Q
1441 AGGTTAAAGTTCAGCGGGATGCCAGGAGAAATGCTGGACATGACGCCGGAGTACGGGCTG
     R  L  K  F  S  G  M  P  G  E  M  L  D  M  T  P  E  Y  G  L
1501 ACCATGAAGCACAAGCGCTGTCTGCTGCGGGTCACGCCGCAACCCGGCTTCGAGCTCCGC
     T  M  K  H  K  R  C  L  L  R  V  T  P  Q  P  G  F  E  L  R
1561 GGCTCCGCGTCGTGATCCGGACGGAGAATCCTCCATCAGCTCGCAACGCCAAATACTCGA
     G  S  A  S  *
1621 AAGCTGTTTGTATCAGTTCTGGTGCGGTTCTTTCAGCCGTCATAATCTCATGTTCATGTG
1681 TTGTCAGTGCAGACGCACGTCTGTCCGGTTCCGCTCCCTGATGACGGGTCAGCGCGAGCC
1741 TCAGTGCCTTCTGTTCTCATCCGCTCACGCCGGACTGAAGCTTCTGCTCCATTACGATCC
1801 CAAAATGTGATCCACCTGTAAATATTCAGCCATGTGCCTGATCTTGTGTTTGTACAGACG
1861 TTTTTTTGTATTTATCAAGGTGCTTCTGGACATTTGATACGATTCTCCATCAAATTCTCC
1921 TCATGAAACTCTTTATCAGGCTCTTAATGTCTTGTATAATGAAGGATTTTAACGTTCTGA
1981 AGGTGCTTCAATAAATCATTCCTGCCAGATGTGCTCCTCATATCGACAGTTGCGATGTTT
2041 TTTGCGTGACCTATTTATGATTTGCTAAATGGAATAATAAAAACTTTTATGAAAAAAAAA
2101 AAAAAAAAA
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Fig. 1. Representative DNA sequence of the *Zacco platypus CYP1A* gene (GenBank Accession No. **JN648712**). An asterisk (*) indicates a stop codon. Poly (A) signal sequence was underlined.

by a post-hoc multiple comparison (Duncan) test was used to compare each tissues. Student's *t*-test was used to determine significances between exposure group at each time point and control. All data were analyzed using the SPSS statistical package (version 10.0; SPSS Inc., Chicago, IL, USA). Data are expressed as means ± S.D. and differences are considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

We directly sequenced amplified clones of the *Z. platypus CYP1A* gene and submitted the sequence to GenBank (Accession no. JN648712) (Fig. 1). The *Z. platypus CYP1A* gene consisted of 1575 bp of the open reading frame (ORF) which encoding 524 amino acids of putative protein, 106 bp of the 5′-untranslated region (UTR) and 534 bp of the long 3′-UTR. The *Z. platypus CYP1A* transcripts were ubiquitously distributed and showing highest level of expression in the liver followed by gill, kidney and intestine (Fig. 2). Liver is the main site of xenobiotic metabolism and high level of *CYP1A* expression in observed in liver in most of the species (Arukwe, 2002; Lee *et al.*, 2005; Miranda *et al.*, 2006, Kim *et al.*, 2008a, b). Also in fish, gill is the important site of absorption and therefore, activities of cytochrome P450-dependant enzymes are associated with it to handle the toxic chemicals when they enter the system (Evans, 1987; Wilson and Laurent, 2002; Jönsson *et al.*, 2003). These similar patterns of distribution of *CYP1A* are also reported in a few fish species such as hermaphrodite fish, *Kryptolebias marmoratus* (Lee *et al.*, 2005), a yellow catfish, *Pelteobagrus fulvidraco* (Kim *et al.*, 2008a) and a river pufferfish, *Takifugu obscurus* (Kim *et al.*, 2008b). On the other hand, Eye, heart, muscle and skin were weakly expressed. Concerning low level of expression of *CYP1A* in these tissues, to our knowledge both these tissues have rarely been studied in fish as target organs for *CYP1A* induction. Otherwise, in mammals *CYP1A* in skin has been focused primarily because it has a function related to carcinogen metabolism (Reiners *et al.*, 1997; Marston *et al.*, 2001; Smith *et al.*, 2006). In general, the organs actively involved in the response to xenobiotics or those involved in their absorption have high levels of *CYP1A* dependant enzyme activities. While differential expression of the *CYP1A* gene in different tissues has also been reported in

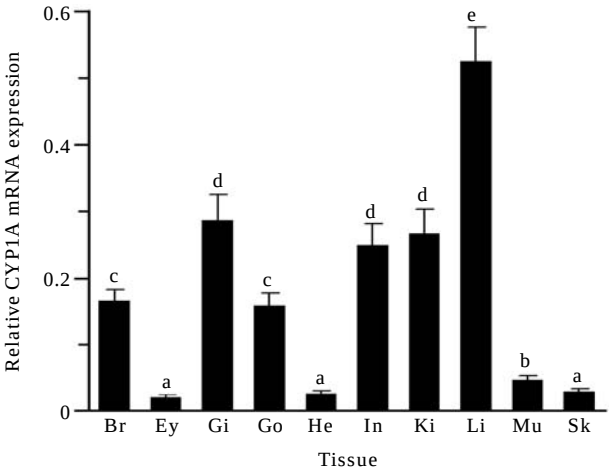

Fig. 2. Expression of *Zacco platypus CYP1A* gene in different tissues (Br: Brain, Ey: Eye, Gi: Gill, Go: Gonad, He: Heart, In: Intestine, Ki: Kidney, Li: Liver, Mu: Muscle, Sk: Skin).

case of Atlantic salmon (*Salmo salar*) by Arukwe (2002) and Rees *et al.* (2003). Thus, the tissue specific induction of *CYP1A* gene expression requires further attention. Hahn (1998a) reviewed the transcriptional regulation of fish *CYP1A* through a transcription factor known as the aryl hydrocarbon receptor (AhR). The transcriptional regulation of *CYP1A* in fish and mammals appears to be similar. *CYP1A* has been studied extensively due to its biological importance in the metabolism and toxicity of various xenobiotics (Hankinson, 1995). *CYP1A* protein expression and activity, although constitutively present at significant levels, are upregulated in response to the binding of AhR agonists. Binding of these agonists to the cytosolic AhR leads to translocation of the AhR to the nucleus, dimerization of the AhR with ARNT ("aryl hydrocarbon receptor nuclear translocator," a misnomer), and subsequent binding to an enhancer region termed the XRE (xenobiotic response element) or DRE (dioxin response element). *CYP1A* is one of several genes having at least one 59 DRE. Also, there is evidence in mammals for regulation of *CYP1A* by other mechanisms by some chemicals (Hoffer *et al.*, 1996; Ledirac *et al.*, 1997). *CYP1A*, a monooxygenase, plays an important role in the Phase-I metabolism of many xenobiotic and endogenous chemicals, including PAHs. We have so far not studied XREs in *Z. platypus CYP1A* but information on this aspect may elucidate the functional properties of the identified genes.

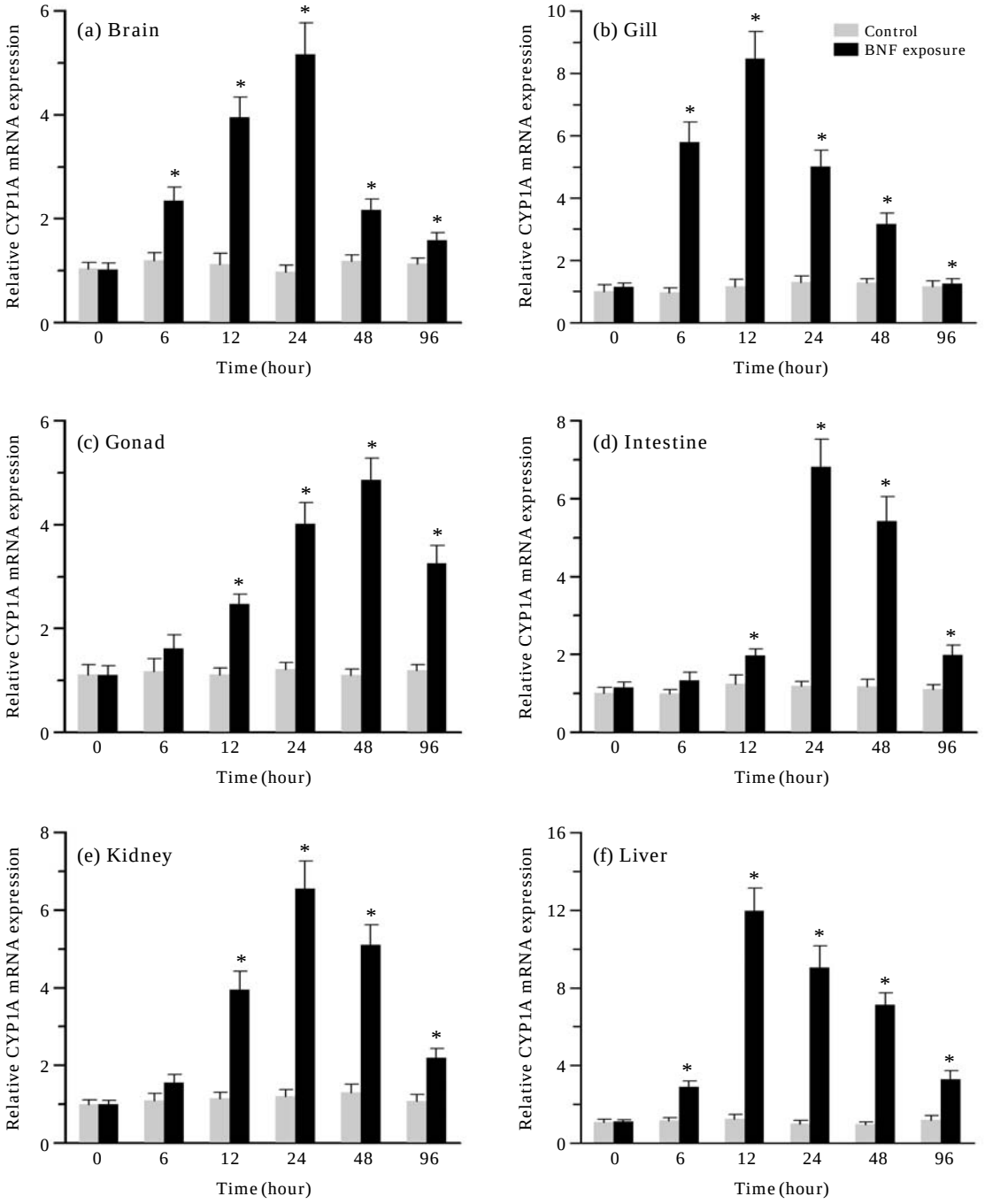

Fig. 3. Relative mRNA expression of *Zacco platypus* CYP1A gene in different tissues after exposure to β-naphthoflavone (1 μM) for 96 hours.

For study of BNF-induced expression of *CYP1A* we selected six vital organs, brain, gill, gonad, intestine, kidney and liver. Time-course studies revealed a different response in all the six tissues (Fig. 3). While brain, gills and liver showed significantly different levels of expression compare with the control level at 6 h ($p<0.05$), the other organs showed a significant induction at 12 h ($p<0.05$). Gill and liver showed the highest level at 12 h, others showed the highest level at 24 h or 48 h. In all the tissues, decreased expression levels were observed at 48 and 96 h when compared

with the levels at 24 h. BNF-induced expression of *CYP1A* has been reported in many fish species (Sarasquete and Segner, 2000; Chung-Davidson *et al.*, 2004; Jönsson *et al.*, 2007; Kim *et al.*, 2008a, b). In contrast to our findings, Chung-Davidson *et al.* (2004) observed that brain *CYP1A* in juvenile trout (*Salvelinus namaycush*) remained induced for an extended period of time. However, similar to our results in brain, gill and liver, *CYP1A* mRNA induction in response to BNF exposure occurred rapidly and continued to rise in the BNF-treated lake trout after 4 h. Because of the large surface area, gills provide a suitable site for absorption of toxicants and therefore role of CYP which catalyzes activation of xenobiotics has been reported from the gills of fish. In our study, gills were highly showed expression of BNF-induced *CYP1A* mRNA. A time-course study also showed high expression during the early phase of exposure (Fig. 3b). In gills of Atlantic salmon (*Salmo salar*), Rees *et al.* (2003) observed a peak response at 6 h and expression levels of gills were even higher than in the liver. In our study kidney of *Z. platypus* also showed appreciable levels of *CYP1A* mRNA expression. However, muscle and skin showed negligible expression. Both these tissues are least important for metabolism of xenobiotics in fish (Monostory *et al.*, 1996).

Overall, this is the first report of a full cDNA sequence of any cytochrome P450 gene from *Z. platypus*. Concerning the fact that *Z. platypus* is ecologically important sentinel fish in vast parts of Asia, further intense studies on molecular gene expression of this fish are needed to be undertaken. Therefore, present study on *Z. platypus* xenobiotic metabolism could be extended to those areas involved in detoxification and antioxidant defense are underway in our laboratory.

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