Molecular Cloning and Expression of Cytochrome P450 1C1 in Japanese Eel (*Anguilla japonica*).

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ABSTRACT

Cytochrome P450 (CYP) enzymes constitute a multigene family of many endogenous and xenobiotic substances. The *CYP1* family is of particular interest in environmental toxicology because its members are dominant in the metabolism of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and aryl amines. A new cDNA of the *CYP1C* subfamily encoding CYP1C1 was isolated from Japanese eel *(Anguilla japonica)* liver after intraperitoneal injection with β-naphthoflavone (BNF). The full-length cDNA obtained (3508 bp) contained a 5' noncoding region of 355 bp, an open reading frame of 1581 bp coding for 526 amino acids, a stop codon, and a 3' noncoding region of 1572 bp. The predicted molecular weight of the protein was approximately 59.33 kDa. The deduced amino acid sequence of Japanese eel *CYP1C1* had the lower similarity of 70% with that of killifish *CYP1C1* while the higher similarity (79 and 81%) was observed with that of rainbow trout *CYP1C2* and *-1C1* sequences respectively. It exhibited similarities of 71% with that of Indian medaka *CYP1C1* and zebrafish *CYP1C2*. Also the similarity of 74% was registered with the sequence of three-spined stickleback fish *CYP1C1*, *-1C2* and carp *CYP1C2*. It showed similarity of 77% with that of Nile tilapia *CYP1C1*, scup *CYP1C1* and scup *CYP1C2*.

The phylogenetic tree showed the newly identified Japanese eel *CYP1C1* sequence to be clustered with rainbow trout *CYP1C1* and *-1C2*. Japanese eel *CYP1C1* was aligned with the *CYP1* sequences and has been deposited in the Gen Bank / EMBL data bank with the accession number AY444748. Quantitative real-time polymerase chain reaction (QRT-PCR) analysis of liver, kidney, intestine and gills revealed a distinct induced expression in all organs studied (283.33, 579.35, 20.96 and 3642.32 respectively).

Keywords: *Anguilla japonica*, β -naphthoflavone, cytochrome P450, *CYP1C1*.

INTRODUCTION

Cytochrome P450 (CYP) enzymes constitute a unique superfamily of heme-containing proteins that are bound to the membrane of the endoplasmic reticulum and play a crucial role as an oxidation-reduction component of the monooxygenase system. In this super family, multiple families and subfamilies are recognized to be active in the oxidative metabolism of a wide range of substrates including drugs and environmental contaminants, as well as endogenous compounds such as steroids, fatty acids, and prostaglandins (Nebert & Gonzalez, 1987; Nelson *et al.,* 1996). The levels of expression of CYP genes in the tissue of fish inhabiting polluted areas have been used extensively in biomonitoring studies as indicators of dioxin pollution. Most chemical carcinogens in the environment are chemically inert and require metabolic

activation by cytochrome P450 (CYP) enzymes to exhibit carcinogenicity in experimental animals and humans (Conney, 1982; Guengerich & Shimada, 1991). Cytochrome P450 enzymes are central to the metabolic activation of PAHs, PCBs, and aryl amines. The CYP1C subfamily of cytochrome P450s, which is present in fish but not in mammals, has only recently been discovered and so limited research on the subfamily is available. The new vertebrate CYP1C subfamily was first described when *CYP1C1* and *CYP1C2* expression were detected in scup *(Stenotomus chrysops)* liver and head kidney (Godard *et al.,* 2005) and *CYP1C1* gill expression was identified in carp (*Cyprinus carpio*) (Itakura *et al.,* 2005). More recently, a full-length *CYP1C1* was cloned and mRNA tissue expression was quantitated by PCR in killifish *(Fundulus heteroclitus)* (Wang *et al.,* 2006) and in *Oreochromis niloticus* (Hassanin *et al.,* 2012) while the induced expression of carp *CYP1C2* was observed in the kidney (Kaminishi *et al.,* 2007). Analysis of sequence domains suggests that fish CYP1B and CYP1C enzymes will almost likely have unique catalytic functions or substrates; however, the function of these newly reported P450s is currently unknown. The common molecular phylogeny from the CYP1 genes in several species thus supports the hypothesis that CYP1Bs and CYP1Cs diverged from a common CYP1B/CYP1C ancestor (Goldstone *et al.,* 2007 & 2009). Two orthologs of *CYP1C1* and *CYP1C2* were found in the fish lineage, however, the CYP1C subfamily was not found in mammals, indicating that this gene was lost in the early mammalian lineage (Godard *et al.,* 2005). Although there has not been a CYP1C identified in mammals; however, because fish are extensively used in toxicology testing, biomonitoring, and as developmental biology and cancer models, it is important to understand the physiological roles, tissue distribution, and metabolic capacity of these *CYP1C* genes. To date, the Genbank survey on another *CYP1C* subfamily, revealed sixteen sequences; *CYP1C1* and *-1C2* sequences from scup, *CYP1C1* and *-1C2* from carp, *CYP1C1* and *-1C2* from zebrafish, *CYP1C1* and *-1C2* from three-spined stickleback fish, *CYP1C1* and *-1C2* from killifish, *CYP1C1* and *-1C2* from rainbow trout, *CYP1C1* and *-1C2* from Indian medaka, *CYP1C1* from Japanese medaka and *CYP1C1* from Nile tilapia.

In this study, a cDNA of the *CYP1C1* gene was isolated from the liver of BNF-treated Japanese eel *(Anguilla japonica)* and sequenced. Phylogenetic analysis was also performed to assess the relationship of this newly identified *CYP1* gene with the other *CYP1* family members and the expression pattern of CYP1C1 mRNA was determined in liver, kidney, intestine and gills of Japanese eel using QRT-PCR.

MATERIALS AND METHODS

Fish treatment

Four Japanese eel *(Anguilla japonica)* weighing about 400 g each were injected intraperitoneally with BNF (100 mg/kg) suspended in corn oil. Samples of the liver, kidney, intestine, and gills of these fish were collected three days after the injection, immediately frozen in liquid nitrogen, and stored at –80 °C. Similar control fish were intraperitoneally injected at the same time with an equivalent volume of the corn oil.

RNA isolation

It is important to optimize the isolation of RNA and to prevent introduction of RNases and inhibitors of reverse transcription (RT). The guanidine isothiocyanate /acid-phenol chloroform method, originally described by Chomzynski & Sacchi, 1978 is the applied method for RNA isolation. Total RNA was isolated from 2 g of frozen liver after lyses in guanidinium salt. The total RNA was quantified

spectrophotometrically based on absorbance at 260 nm as described by Sambrook & Russell, (2001). Poly $(A)^+$ RNA was purified using the Oligotex -dT30 〈Super〉 mRNA Purification kit (Takara, JAPAN) .

Reverse transcriptase-assisted polymerase chain reaction

Reverse transcription (RT) of mRNA was performed with Superscript II reverse transcriptase (Gibco BRL, USA) to generate 5'-RACE-Ready and 3'-RACE-Ready first-strand cDNAs using the SMART^{TM} RACE (rapid amplification of cDNA ends) cDNA amplification kit (Clontech, USA) according to the manufacturer's protocol.

3'and 5'–RACE PCRs for full-length cDNA

We designed one sense (F) and an antisense (R) primer specific to Japanese eel CYP1C1 for 3' and 5' RACE PCRs, respectively. Primer sequences are given in Table 1. The sense and antisense gene specific primers were used in combination with the universal primer mix (UPM) of the RACE PCR kit to generate the RACE PCR products using the SMART RACE cDNA Amplification kit (Clontech, USA). The cycle conditions for the RACE PCR were as follows: 5 cycles of denaturation for 5 s at 94 °C and annealing for 3 min at 72 °C; 5 cycles of 94 °C for 5 s, 70°C for 10 s, and 72 °C for 3 min; and 35 cycles of 94 °C for 5 s, 68 °C for 10 s, and 72°C for 3 min. For cloning, DNA bands were excised from the gel and extracted using a GFX PCR DNA and Gel Band Purification kit (Amersham Biosciences, USA), the PCR products were subjected to restriction mapping with various enzymes and subcloned into the pBluescript II SK(+) vector (MBI Fermentas, USA). Purified plasmids were directly sequenced by dye terminator cycle sequencing using the ABI PRISM Dye Terminator Cycle Sequencing kit (PE Biosystems, USA) and an Applied Biosystems 3100 DNA Sequencer.

Table 1: Oligonucleotide primers used in the PCR amplification of Japanese eel CYP1B1 cDNA fragments.

Primer	Nucleotide sequence	Nucleotide location
F	5'- CTTGTCATTCAAGGCAAGCAAAGAC	1963 to 1987
R	5'-AAACCATGTGAGAGCCCTGAAACT	1197 to 1220
UPM (long)	5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	
UPM (short)	5'-CTAATACGACTCACTATAGGGC	

Phylogenetic analysis

DNA sequences with the following GenBank accession numbers were retrieved from the database and used in the phylogenetic analysis: AY437776 (carp *CYP1C1*), AF131885 (scup *CYP1C1*), JX454611 (Indian medaka *CYP1C1*), EF54668 (Japanese medaka *CYP1C1*), NM_001185031 (rainbow trout *CYP1C1*), NM_001267692 (three-spined stickleback fish *CYP1C1*), DQ133570 (killifish *CYP1C1*), NM_001279575 (Nile tilapia *CYP1C1*), AY928186 (zebrafish *CYP1C1*), AY437777 (carp *CYP1C2*), DQ133571 (killifish *CYP1C2*), AF235138 (scup *CYP1C2*), JX454612 (Indian medaka *CYP1C2*), HQ202284 (three-spined stickleback fish *CYP1C2*), NM_001185032 (rainbow trout *CYP1C2*) and BC095332 (zebrafish *CYP1C2*). In order to determine homology among CYP1C subfamily cDNAs or deduced amino acid sequences of various species, sequence alignments were performed by the CLUSTALW multiple sequence alignment method (online alignment site constructed by Kyoto University, http://www.genome.jp/tools/clustalw/) or with laser gene DNASTAR program (Ver. 5.52, 2003, DNASTAR Inc). The phylogenetic tree was constructed by unweighted pair group method (UPGM) using the amino acid sequences of the previously reported CYP1C subfamily sequences.

CYP1C1 expression profile in different organs of Japanese eel using QRT-PCR reverse transcription

Reverse transcription of the RNA samples isolated from liver, kidney, intestine and gills was performed using Primescript^{TM} RT reagent kit (Takara, Japan) according to the manufacturer's instructions. Reactions were incubated for 15 min at 37°C then 5 s at 85°C to inactivate the reverse transcriptase. RT products were stored at 4°C for further PCRs.

Primer design for QRT-PCR reaction

Primers for *Japanese eel CYP1C1* cDNA were designed using Laser gene primer select program (Ver. 5.52, 2003, DNASTAR Inc), with melting temperatures (Tm) ranging from 58 to 60°C, and amplicon lengths of 50 to 150 bp. The optimal annealing temperatures were close enough to run all reactions under the same thermal parameters. The primer sequences are given in Table (2).

Table 2: Oligonucleotide primers used in the QRT- PCR amplification of Japanese eel probe.

Primer	Nucleotide sequence	Nucleotide location	Product length
F	5'- AGTGTTCCTTGTGGGGTGGTGAGA	2206 to 2229	107bp
R	5'- AAACCATGTGAGAGCCCTGAAACT	783 to 806	

QRT-PCR conditions and analysis

Each PCR reaction consisted of 10 µl of SYBR^{R} Premix Ex Taq^{TM} II (2X), 10 µM of each primer, 2 µl of cDNA template and double distilled water to a final volume of 20 µl. All standard plasmid DNA dilutions, template controls and induced samples were run in triplicates. Reactions were then analyzed on an ABI 7300 Real-Time PCR system under the following conditions: 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 3 min. In the relative standard curve method, the relative quantification relates the PCR signal of the target transcript in a treatment group to that of another sample such as an untreated control (calibrator). For quantification of induced Japanese eel CYP1C1 normalized to an untreated control, standard curves were prepared for both the induced and the untreated reference. Each of the normalized induced Japanese eel CYP1C1 values was divided by the untreated control normalized value to generate the relative expression levels. Accordingly, CYP1B1 mRNA levels were reported as fold change in abundance relative to the average calibrator response.

Statistical analysis

The statistical differences between the groups were determined, and the data expressed as mean ± standard deviation. Excel (Microsoft, NY) were used to analyze the data, and Student's t test was used for the comparisons. A P-value <0.05 was considered significant. At least three determinations were carried out for each data point.

RESULTS

Nucleotide sequence analysis

Figure 1 shows the full nucleotide sequence (3508 bp) contained a 5' noncoding region of 356 bp, an open reading frame of 1581 bp coding for 526 amino acids, a stop codon, and a 3' noncoding region of 1572 bp. The predicted molecular weight of the protein was approximately 59.3 kDa. The sequence had four polyadenylation signal (AATAAA) and a poly A tail of 30 nucleotides. This sequence was aligned with the *CYP1* sequences by the CLUSTAL W method using the Lasergene Megalign program (Ver. 5.52, 2003, DNASTAR Inc) and has been deposited in the

GenBank/EMBL data bank with the accession number AY437776.

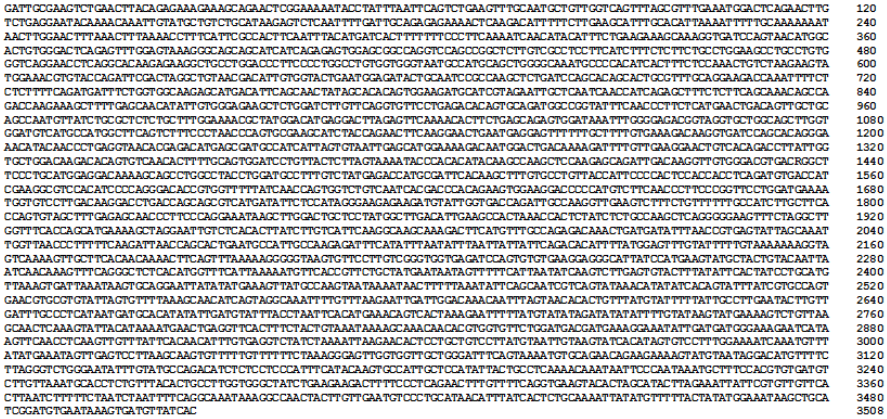

Fig. 1: Nucleotide sequence (3508 bp) of Japanese eel cytochrome P450 CYP1C1 cDNA. Consensus sequences for polyadenylation signals are bolded.

Comparison of amino acid sequences

The open reading frame and its deduced amino acid residues of Japanese eel cytochrome P450, *CYP1C1* cDNA is shown in Figure 2. Table 3 shows the percent similarities of the deduced amino acid sequences of Japanese eel *CYP1C1* with those of the other *CYP1C* subfamily members. The deduced amino acid sequence of Japanese eel *CYP1C1* had the lower similarity of 70% with that of killifish *CYP1C1* while the higher similarity (79 and 81%) was observed with that of rainbow trout *CYP1C2* and *-1C1* sequences respectively. It exhibited similarities of 71% with that of Indian medaka *CYP1C1* and zebrafish *CYP1C2*. Also the similarity of 74% was registered with the sequence of three-spined stickleback fish *CYP1C1*, *-1C2* and carp *CYP1C2*. It showed similarity of 77% with that of Nile tilapia *CYP1C1*, scup *CYP1C1* and scup *CYP1C2*.

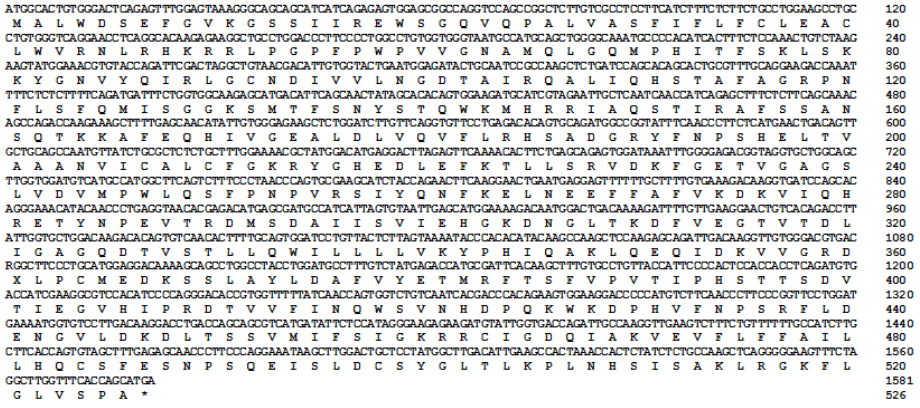

Fig. 2: The open reading frame (ORF) and its deduced amino acid sequence of Japanese eel cytochrome P450 CYP1C1 cDNA.

Table 3: Percent identities of deduced amino acid sequences of CYP1C subfamily genes

	CYP1C1								CYP1C2						
	carp	I. medaka	J. medaka	killifish	zebrafish	three -spined	rainbow trout	Nile tilapia	scup	I. medaka	carp	scup	killifish	zebrafish	three -spined	rainbow trout
Japanese eel 1C1	76	75	75	73	75	74	81	77	77	71	74	77	70	71	74	79
carp 1C1		72	73	72	84	73	76	76	77	70	78	73	68	76	71	73
Indian medaka 1C1			91	80	72	81	78	82	82	73	70	77	70	67	75	75
Japanese medaka 1C1				80	71	80	78	83	82	72	72	78	70	66	75	76
killifish 1C1					72	79	75	81	80	71	70	75	70	68	74	73
zebrafish 1C1						74	75	74	76	70	75	73	67	79	71	73
three-spined 1C1							76	83	87	73	71	76	69	69	79	73
rainbow trout 1C1								78	79	72	73	76	71	71	73	85
Nile tilapia 1C1									86	72	72	78	69	76	74	74
scup CYP 1C1										74	72	81	71	71	77	75
Indian medaka 1C2											71	78	78	69	78	75
carp 1C2												74	68	83	72	75
scup CYP1C2													78	73	85	79
killifish 1C2														69	77	74
zebrafish 1C2															71	73
three-spined 1C2																77

Phylogenetic analysis

The phylogenetic tree (Figure 3) based on the amino acid sequences of CYP1C subfamily species showed a closer relationship of the newly identified Japanese eel *CYP1C1* sequence to that of rainbow trout *CYP1C1* and *-1C2*.

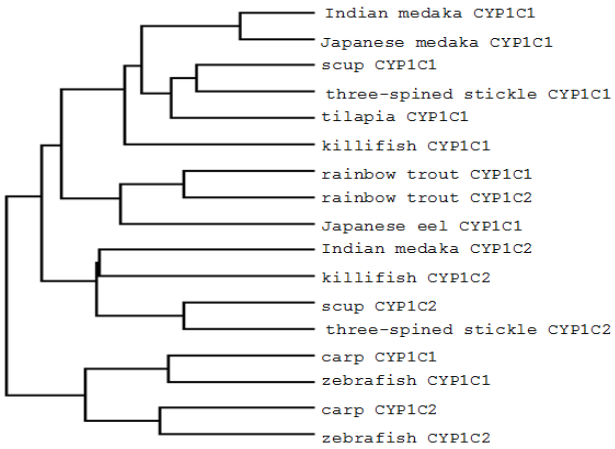

Fig. 3: Phylogenetic tree of CYP1C subfamily genes constructed by the unweighted pair group method (UPGM) using the amino acid sequences of teleosts.

CYP1C1 mRNA level in different tissues of BNF treated Japanese eel

QRT-RCR results revealed that there was a large increase in CYP1C1 mRNA in gills (3642.32 fold), followed by kidney (579.35), liver (283.33) and intestine (20.96) (Table, 4) (Figure 4).

Table 4: QRT-PCR results for Japanese eel CYP1C1 mRNA

Sample	Mean Qty	Std Dev	Fold increment	t-value	Pr
L.cont	0.0132	0.00		4.94	<0.01
L.ind	3.74	1.31	283.33		***
K.cont	0.6825	0.2937		3.58	<0.025
K.ind	395.405	191.18	579.35		*
G.cont	1.81	0.6264		11.71	<0.005
G.ind	6592.6	974.54	3642.32		***
I.cont	14.178	3.227		5.04	<0.01
I.ind	297.23	97.165	20.96		***

L. cont = Liver control; L. ind = liver induced; K. cont = kidney control; K. ind = kidney induced; G. cont = gill control; G. ind = gill induced; I. cont = intestine control; I. ind = intestine induced.

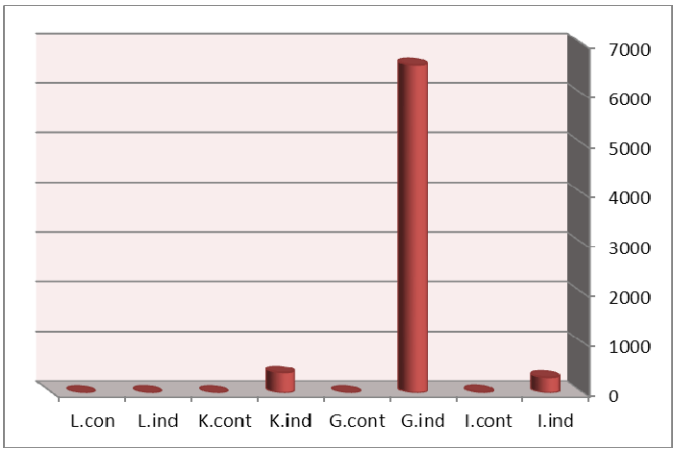

Fig. 4: The expression pattern of CYP1C1 in different organs of BNF-treated Japanese eel compared to the control; L.cont = Liver control; L.ind = liver induced; K.cont = kidney control; K.ind = kidney induced; G.cont = gill control; G.ind = gill induced; I.cont = intestine control; I.ind = intestine induced.

DISCUSSION

The full-length cDNA obtained of Japanese eel *CYP1C1* was 3508 bp long with an open reading frame of 1578 bp which encodes for 526 amino acids as previously reported with tilapia *CYP1C1* (Hassanain *et al.,* 2012). The other studies on CYP1C subfamily sequences resulted in a slight change in the number of amino acids. Zebrafish showing 523 amino acids (Hou-Chu Yin *et al.,* 2008) while carp showing 524 amino acids (El-Kady *et al.,* 2004a, b). Scup *CYP1C1* has an open reading frame of 1575 bp which encodes a predicted protein of 525 amino acids long while scup *CYP1C2* has an open reading frame of 1569 bp that encodes for 523 amino acids (Godard *et al.,* 2005).

The present study on Japanese eel *CYP1C1,* revealed the higher expression pattern of mRNA in gills (3642.32), followed by kidney (579.35), liver (283.33) and intestine (20.96). However, provided that the Japanese eel *CYP1C1* cDNA was obtained from the liver, the low induction pattern in the liver, kidney, and intestine may suggest the possibility of low levels of the *CYP1C1* gene in these organs. Godard *et al.,* 2005 found that CYP1Cs are expressed in liver and head kidney of untreated male scup; the expression levels were higher in liver than in head kidney. In contrast to scup, Wang *et al.,* 2006 stated that *CYP1C1* expressed more highly in kidney of killifish following a 15-day waterborne BaP exposure. They reported the highest *CYP1C1* constitutive expression in spleen, kidney, eye, gill and gonad, respectively.

In our previous study using Northern blot analysis, the carp *CYP1C1* was not induced in liver, intestine, or kidney but the constitutive expression was observed in gills 24 h following injection of BNF (Itakura *et al.,* 2005). Another study on *CYP1* genes of killifish revealed the highest expression levels of both *CYP1C1* and *CYP1C2* in liver, gills and kidney (Zanette *et al.,* 2009). The author reported the higher expression of *CYP1C1* in testis ~1000 and 3000 times more than *CYP1A* and *CYP1B1*, respectively while *CYP1C2* was expressed at the lowest levels among the five *CYP1* genes in most of the organs examined (liver, heart, kidney, eye, brain and kidney). Jönsson *et al.* (2010) reported that all transcripts of CYP1 family were induced by PCB126 in gills and liver of rainbow trout, suggesting all genes to be AhR regulated. The caged fish showed clear rbCYP1 induction in gills at all monitoring

sites (up to 70-fold the basal level), whereas the liver responses were weak; induction (up to 5-fold). Dorrington *et al.* (2012) reported that 3-MC induced *CYP1A*, *CYP1B1*, and *CYP1C1* significantly (20–120-fold) in the liver, gill and intestine of Brazilian guppy. In a study of the effects of effluent from drug manufacturing on the cytochrome P4501 regulation and function in fish, Beijer *et al.,* 2013 reported the induction of *CYP1B1* and *CYP1C1* mRNA of three-spined stickleback fish *(Gasterosteus aculeatus)* in gills at all concentrations while effects on these genes in liver and brain were weak or absent. Another study on three-spined stickleback fish exposed to a transient and persistent inducers, reported the *CYP1C1* transcript was most highly expressed in the brain and showed no difference in expression level in the other studied organs (brain>liver=gill=kidney) (Gao *et al.,* 2011). The author recorded the dominant expression of *CYP1C2* in the kidney (kidney>brain=liver>gill). Real time PCR results revealed the large increase in *CYP1C1* mRNA in liver (43.1), intestine (5.1) and muscle (2.4) of Nile tilapia 24 h following a 100 mg/kg intracoelomic injection of Bap (Hassanain *et al.,* 2012).

In our previous studies, the constitutive expression in the gill organs was observed in the 3-MC of carp *CYP1B1* which showed the induced expression in the liver and intestine (El-kady *et al.,* 2004a), while carp *CYP1B2* was induced by 3-MC in the gills but not in the liver or intestine with no detectable constitutive expression in the organs examined (El-kady *et al.,* 2004b). The restricted tissue expression in the gills was reported by *Leaver and George, (2000*) who studied the plaice *CYP1B1* gene (called *CYP1B* in the report) using total RNA in Northern blot analysis. On the other hand, the similar expression patterns of CYP1B/1C genes in gills of carp, plaice, Brazilian guppy, three-spined stickleback, rainbow trout and Japanese eel may suggest that endogenous functions of these genes may be served similarly in different vertebrate groups.

CONCLUSION

This study provides the first description of the molecular cloning of Japanese eel CYP1C1 and the relationship of this newly identified sequence with that of the previously reported CYP1C subfamily members. Also the higher induction of Japanese eel CYP1C1 mRNA in gills (3642.32), followed by kidney (579.35), liver (283.33) and intestine (20.96) may have important implication for furthering our understanding of the possible CYP1C1 functions in these organs.

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ARABIC SUMMARY

الاستنساخ الجزيئى وقياس التعبير الجينى للسيتوكروم P450 1C1 فى ثعبان السمك اليابانى *Anguilla (japonica)*

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تشكل عائلة السيتوكروم P4501 مجموعة كبيرة من الإنزيمات متعددة الجينات . وعائلة CYP1 هى موضع اهتمام خاص في علم السموم البيئية لأن أعضائها هي السائدة والمسؤولة عن أيض الملوثات الكيميائية مثل المواد الهيدروكربونية العطرية متعددة الحلقات ، ثنائي الفينيل متعدد الكلور و أريل الأمينات. فى هذه الدراسة تم عزل الحمض النووي التكميلى cDNA من فصيلة CYP1C وتم تسميته CYP1C1 وذلك من كبد ثعبان السمك الياباني بعد الحقن داخل الغشاء البريتونى بمادة β – naphthoflavone، وكان طول هذا cDNA هو(٣٥٠٨ bp) تحتوي على ٥ ' غير المكودة مكونه من ٢٤٤ bp ويحتوي على منطقة ORF مكونة من١٥٨١ bp التى وتشفر لـ ٥٢٦ حمضا أمينيا والوزن الجزيئ الناتج لهذا البروتين حوالي ٥٩,٣٣ كيلو دالتون . أظهر تسلسل الأحماض الأمينية لجينCYP1C1 المعزول من ثعبان السمك اليابانى ٧٠٪ تشابها للجين CYP1C1المعزول من أسماك الكيلي فيش CYP1C1 في حين لوحظ أعلى نسبة تشابه (٧٩ و ٨١٪) مع أسماك تراوت قوس قزح CYP1C2 و1C1 على التوالي. بينما أظهرت نسبة تشابه ٧١٪ مع CYP1C1 المعزول من الميداكا الهندية وCYP1C2 لأسماك الزرد. كما تم تسجيل ٧٤٪ من التشابه مع CYP1C1 لأسماك three-spined stickle back، 1C2 والكارب CYP1C2. كما أظهرت نسبة تشابه ٧٧٪ مع أسماك البلطي النيلي CYP1C1، CYP1C1 scup وCYP1C2 scup.

كان نتيجة التشابه الكبير (٨١ و ٧٩ %) بين الجين المعزول من ثعبان السمك اليابانى وأسماك التراوت قوس قزح CYP1C1 و CYP1C2 عن وجود هذه الجينات على فرع واحد باستخدام شجرة النشوء والتطور. تم محاذاة الجين المعزول من ثعبان السمك اليابانى CYP1C1 مع جينات عائلة CYP1 وأودع في بنك الجينات EMBL تحت رقم AY444748. وكشف تفاعل البلمرة المتسلسل الكمى (QRT-PCR) لتحليل الكبد والكلى و الأمعاء و الخياشيم تعبيراً جينيا ملحوظا ومستحثا بلغت قيمته (٢٨٣,٣٣ ، ٥٧٩,٣٥ ، ٢٠,٩٦ و ٣٦٤٢,٣٢ على التوالي) .