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Biochemical Pharmacology 63 (2002) 767–775

Biochemical Pharmacology

Induction of UDP-glucuronosyltransferase 1A8 mRNA by 3-methylcholanthene in rat hepatoma cells

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Received 1 July 2001; accepted 13 November 2001

Abstract

UDP-glucuronosyltransferases (UGTs) catalyze the glucuronidation of a broad spectrum of endobiotic and xenobiotic compounds, which leads to the excretion of hydrophilic glucuronides via bile or urine. By a mechanism of exon sharing, isoforms of the UGT1 family are made from the complex gene locus by an alternative combination of one of the unique first exons with the other commonly used exons. This study demonstrates that the expression of the UGT1 gene *UGT1A6*, *1A7* and *1A8* is regulated at the transcriptional level by 3-methylcholanthene (3-MC) in rat hepatoma H-4-II-E cells. Following 3-MC treatment, there is a gradual increase in the amount of UGT1A6 and UGT1A7 mRNA to the maximum levels after 16 hr of treatment. The induction effect of 3-MC led to the expression of UGT1A8 which has not been reported before. This induction is suppressed by the RNA synthesis inhibitor actinomycin D, indicating that the inducer does not act at the level of mRNA stabilization. Northern blot analysis showed a 4-fold increase in UGT1A8 transcription after treatment with 3-MC. The prolonged treatment with the protein synthesis inhibitor did not affect the induction process. The results provide experimental evidence for a transcriptional control of UGT1A8 synthesis. Transcriptional activation of the UGT1A8 by 3-MC does not appear to require *de novo* protein synthesis. 3-MC dependent activation is probably the result of a direct action of the compound on the aryl hydrocarbon receptor complex (AhR).

Keywords: UGT1A8; Induction; Transcription; Expression

1. Introduction

Glucuronidation is a major detoxification pathway in mammals. Glucuronide formation is catalysed by a family of UDP-glucuronosyltransferases (UGTs) [1], with a wide spectrum of endobiotic and xenobiotic compounds as substrates [2,3]. The regulation of UGTs plays an important role in detoxification of xenobiotics [4], including drugs, carcinogens, environmental pollutants, steroids, bile acids and bilirubin. Transfer of the glucuronic acid moiety to the substrate results in a more water-soluble glucuronide and subsequently facilitates its excretion via urine, bile and feces. Based on amino acid sequence homology, UGTs have been divided into two families, UGT1 and UGT2 [1]. UGT2 is composed of multiple steroid UGTs with broad substrate specificity, while *UGT1* are encoded as a large gene cluster. This gene cluster consists of multiple exons 1 and one set of exons 2–5 [5]. The variable first exon codes for the N-terminus of the proteins, which determine substrate specificity. Different UGT1 isozymes result from differential promoter usage and thus from splicing of different first exons to the common exons 2–5 [6]. At least 13 different exons 1 have been identified in the humans while 9 exons 1 have been reported in the rat gene complex [5,7,8].

In the rat, the *UGT1A* gene locus has been cloned and nine different first exons have been sequenced [8]. The corresponding UGT1A proteins are designated UGT1A1 through UGT1A9 [1]. Seven rat first exons contain intact open reading frames, while the first exons coding for UGT1A4 and UGT1A9 contain premature stop codon [8]. Individual UGT1A first exons are preceded by promoter sequences, suggesting a tissue-specific expression and individual indelibility of each *UGT1A* gene [9]. The differential regulation of gene expression by xenobiotics and endobiotics has not been well characterized. UGT1A6, a phenol glucuronidating UGT, was reported to be

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Abbreviations: UGT, UDP-glucuronosyltransferase; AhR, aryl hydrocarbon receptor; 3-MC, 3-methylcholanthrene; XRE, xenobiotic responsive element.

0006-2952/02/$ – see front matter
PII: S0006-2952(01)00902-9

expressed in liver, kidney, lung, spleen, intestine, testis, pyramidal cells and astrocytes of the brain [8,10–12] while UGT1A1 and 1A7 were expressed in the liver, kidney and the gastrointestinal tract [13]. UGT1A6 is inducible by polycyclic aromatic hydrocarbons (PAH) such as 3-methylcholanthrene (3-MC) [8,9,11,14]. UGT1A7 has recently been described as a second 3-MC inducible UGT [15] which mediates the glucuronidation of the carcinogen metabolite benzo[α]pyrene-7, 8-diol [16]. Little is known about the expression of UGT1A2, UGT1A3, UGT1A5 and UGT1A8 in rats. Expression and inducibility of these UGT1A mRNAs in uninduced rat livers show no signals for these corresponding UGTs [8]. After treatment with clofibrate, UGT1A1, UGT1A2 and UGT1A5 were expressed at low levels while treatment with dexamethasone yielded a minor induction of UGT1A2 and UGT1A5 in hepatic tissue [8]. The molecular basis and the physiological significance of UGTs remain to be elucidated.

In the present study, the effects of 3-MC on the individual UGT1A mRNA in rat liver cells were analyzed by RT-PCR and Northern blots. In addition, the regulation of UGT1A8 was characterized by 3-MC induction.

2. Materials and methods

2.1. Cell culture

The rat hepatoma cell line (H-4-II-E) was purchased from ATCC. The cells were cultured in Modified Eagle's Medium supplemented with 5% fetal bovine serum and 5% calf serum at 37° in a 5% CO_2 incubator.

2.2. Chemical treatment

H-4-II-E (3×10^5) were seeded in six-well culture plates and allowed to attach for 48 hr before drug treatment. 3-MC was dissolved in dimethylsulfoxide whereas other drugs were dissolved in complete medium. To determine the effects of 3-MC on UGT1A family expression, different concentrations (0.625–10 μM at final concentration) of 3-MC were chosen for analysis and the cells were harvested at various time points (8, 16, 24 and 48 hr). To study the effects of actinomycin D and cycloheximide on UGT1A isozymes expression, 5 μg/mL actinomycin D or 10 μg/mL cycloheximide in the absence or presence of 10 μM 3-MC were added to the cultures. Cells were harvested after 6 or 16 hr of treatment. A control was setup with culture medium and 0.5% dimethylsulfoxide only.

2.3. RNA isolation

Total RNA was extracted using Trizol reagent (Gibco BRL). RNA concentration was determined by spectrophotometry and RNA integrity was visualized by agarose gel electrophoresis.

2.4. RT-PCR analysis

Complementary DNA was synthesized for RNA samples by mixing 5 μg of total RNA, 500 ng of oligo dT primer, 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiotheitol, 500 μM of each dNTP and 200 U of Superscript II (Gibco BRL) in a total volume of 20 μL. Reverse transcription was performed at 42° for 50 min. Samples were denatured for 15 min at 70° and chilled on ice. PCR reactions were performed in a final volume of 50 μL in a GeneAmp 9700 thermal cycler (Perkin-Elmer). Each PCR reaction contained 0.5 μL of cDNA, 20 mM Tris–HCl, pH 8.4, 50 mM KCl, 1 mM dNTP mixture, 1.5 mM $MgCl_2$ and 0.25 μM primer of the UGT family 1 and 2.5 U of recombinant Taq polymerase (Gibco BRL). The PCR mixture was incubated at 94° for 3 min followed by 25–35 cycles of amplification. Each cycle consisted of 1 min of denaturation at 94°, 1 min of annealing at 60–65° and 2 min of extension at 72°. After the reaction cycles were completed, a final extension step at 72° for 10 min was performed. To evaluate the efficiency of each RT reaction, PCR with rat β-actin specific primers was conducted. Primers used for the study of UGT1A expression by RT-PCR were followed based on Emi method [8] with modifications: five exon 1 specific forward primers: UGT1A1 forward primer (accession no. d38065) 5′-TGG TGT GCC GGA GCT CAT GTT CG-3′; UGT1A5 forward primer (accession no. d38069) 5′-ATG TGA CCC TGC AAG GAT TAG CTG G-3′; UGT1A6 forward primer (accession no. d38061) 5′-TGC TCG ACT TCC TGC AGG CTT TC-3′; UGT1A7 forward primer (accession no. d38061) 5′-CAG TTG GCA GCT GGG AAA ACC A-3′; UGT1A8 forward primer (accession no. d38062) 5′-ACC CTC CCT TCC TCT GTG TGT T-3′ with a common reverse primer on exon 5 (accession no. u20551) 5′-TCA GTG GGT CTT GGA TTT GTG-3′.

2.5. cDNA probes preparation

β-Actin DNA probe was synthesized by PCR as described previously. To maximize the specificity of UGT1A8 DNA probe, PCR was carried out with another set of primer: forward primer 5′-CTG AGT CAC AGA GGG CAT GA-3′ and reverse primer: 5′-CAG CAT-CAACG AAC TCT GC-3′. Amplified cDNAs corresponding to the expected 292 bp fragment of UGT1A8 exon 1 and 232 bp fragment of β-actin were gel purified by nucleospin column (Clontech).

2.6. Northern blot analysis

Ten micrograms of total RNA isolated from cell culture were separated on a 1.5% agarose gel containing formaldehyde and transferred to Hybond N^+ nitrocellulose membranes (Amersham) using 3 M sodium chloride and 0.15 M sodium citrate (20× SSC). The membranes were exposed

to UV by UVP crosslinker (UVP). Fifty nanograms of each cDNA were labeled with ^{32}P dCTP using the Rediprime DNA labeling system (Amersham) according to the supplier's instructions. The membranes were then hybridized for 2 hr at 65° with Rapid-hyb buffer (Amersham) plus the appropriate cDNA probe. After the hybridization was completed, the membranes were washed once in 2× SSC with 0.1% SDS at 65° for 20 min with constant agitation, twice in 0.2× SSC with 0.1% SDS at 65° for 15 min and once in 2× SSC at room temperature for 5 min and subjected to autoradiography at −70° for 7 days. The relative amount of 1A8 mRNA was estimated by densitometric scanning of the Northern blot autoradiograms. The blots were rehybridized with a β-actin probe and the amount of actin mRNA in each lane was used as a standard to quantify UGT1A8 mRNA.

2.7. The 5′ flanking region analysis of UGT1A8

Genomic DNA was isolated from H-4-II-E cell culture. Five micrograms of DNA were incubated with 10 U of *Not*I (Roche) at 37° for 16 hr. The DNA was then purified by nucleospin column (Clontech). Homopolymeric tail of dC was added to 3′ end of 1 μg DNA by 50 U of terminal transferase (Roche) with the buffer conditions: 200 mM potassium cacodylate, 25 mM Tris–HCl (pH 6.6), 0.25 mg/mL bovine serum albumin, 0.75 mM cobalt chloride and 5 μM dCTP. The reaction mixture was then incubated at 37° for 15 min. After the reaction was completed, the dC-tailed DNA was purified and subsequently used for several rounds of PCR reactions. For the first round PCR, 200 nM of G-rich forward primer (5GR-DW) and 200 nM of gene-specific primer (GSP2-2 at +528) were used.

5GR-DW: 5′-GCT GTC AAC GAT ACG CTA CGT AAC GGC ATG ACA GTG GGI IGG GII GGG II-3′; GSP2-2: 5′-CAC TGG GCA CCC TCA TCA AG-3′; for the nested PCR, two forward primers (GR and GR2) and three gene-specific primers (GSP1-2 at +330, GSP2-1 at +188 and GSP1-1 at +43) were added for further analysis.

GR: 5′-GCT GTC AAC GAT ACG CTA CGT AAC G-3′; GR2: 5′-CGC TAC GTA ACG GCA TGA CAG TG-3′; GSP1-2: 5′-CGA AGA AAC CTT TGC CTG AAT GC-3′; GSP2-1: 5′-CGA CTT TCC CAT GTG CCA AC-3′; GSP1-1: 5′-GCC AGA GGC CAG GAA CAG AC-3′.

PCR was carried out with touchdown conditions: (1) 94° for 2 min; (2) five cycles of 94° 30 s and 72° 1 min; (3) five cycles of 94° 30 s, 70° 30 s and 72° 1 min; (4) five cycles of 94° 30 s, 68° 30 s and 72° 1 min. The annealing temperature was then stepped down by two for the next five cycles. When the annealing temperature reached 60°, forty more cycles were carried out for amplification and the final extension step (72° for 10 min) was performed at the end of last cycle. The PCR products were purified and ligated to PCR2.1 vector (Invitrogen) by 1 U of T4 DNA ligase (Roche) with the buffer conditions of 50 mM Tris–HCl (pH 7.6), 10 mM dithioerythitol and 500 μg/mL bovine serum albumin. The reaction mixture was incubated at 4° for 16 hr. The vector was transformed into TOP10 cells (Invitrogen) and the subsequent steps were based on the manufacturer's instruction. PCR was performed to select the colonies which showed the expected size of PCR products by using M13 reverse and T7 primers. Then the colonies were selected to grow overnight. The plasmids were isolated by Quantum Prep Plasmid Miniprep Kit (Bio-Rad) and finally subjected to automatic DNA sequencing (LICOR). The identity of the sequences was confirmed by BLAST software in National Center for Biotechnology Information (NCBI) and Clustalw in European Bioinformatics Institute (EBI). The possible binding elements located in the 5′ upstream region of UGT1A8 were analysed by Genomatix MatInspector professional 4.3 software (http://genomatix.gsf.de) [17] and Transcription Element Search System (TESS, http://www.cbil.upenn.edu/tess).

2.8. Mapping of transcription initiation by 5′ rapid amplification of cDNA end (5′ RACE)

Reverse transcription was first carried out to generate gene-specific cDNA by using 2.5 pmol of GSP2-2. The cDNA was purified. Homopolymeric tail of dC was added to 3′ end of the cDNA by terminal transferase (Roche) as mentioned previously. A series of touchdown PCR were performed using same primers and PCR conditions as described in 5′ flanking region analysis.

3. Results

3.1. Induction of UGT1A mRNA by 3-MC

The rat hepatoma cell line was treated with different concentrations of 3-MC for different periods of time. Fig. 1 shows that the levels of UGT1A6, UGT1A7 and UGT1A8 mRNA increased at different time points after 3-MC treatment using the β-actin mRNA levels as the reference. 3-MC led to a stronger increase of the UGT1A6 and UGT1A7 mRNA levels, than that of UGT1A8 after 3-MC treatment for 6 hr. The induction level of UGT1A8 gradually increased with time and reached the maximum after 24 hr incubation (Fig. 1). Following treatment with 3-MC, the levels of the UGT1A6, UGT1A7 and UGT1A8 transcripts showed 2–4-fold increase upon normalization with the β-actin level while UGT isozyme 1A1 and 1A5 did not show change in the mRNA levels (Fig. 2). The increase of UGT1A6 and UGT1A7 transcripts are in agreement with the quantitative RT-PCR analysis described earlier [13,18] and correlate well with increase in both the UGT1A6 and UGT1A7 protein levels upon treatment with 3-MC. These results provide evidence that 3-MC is able to increase UGT1A6, 1A7 and 1A8 mRNA levels in an inducer-specific and time-dependent manner.

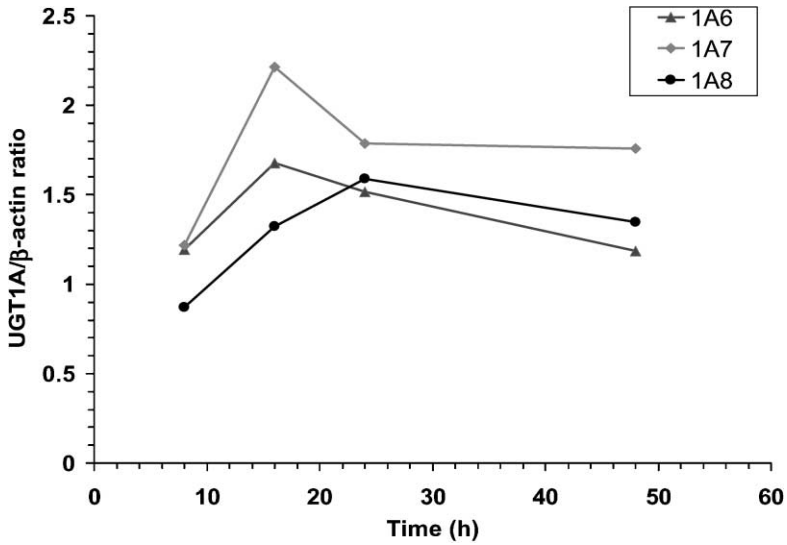

Fig. 1. Time-course study of the expression levels of UGT1A6, 1A7 and 1A8 after treatment with 10 μM 3-MC.

3.2. Transcriptional induction of UGT1A8 expression by 3-MC

The relative amount of UGT1A8 mRNA was assayed by Northern blot analysis. The radiolabeled cDNA probes were synthesized and hybridized to UGT1A8 and β-actin cDNA. Hybridization of mRNA to the UGT1A8 cDNA probe revealed a prominent transcript of about 2.5 kbp (Fig. 3). Northern blot analysis showed that 3-MC treatment augmented the levels of UGT1A8 transcripts relative

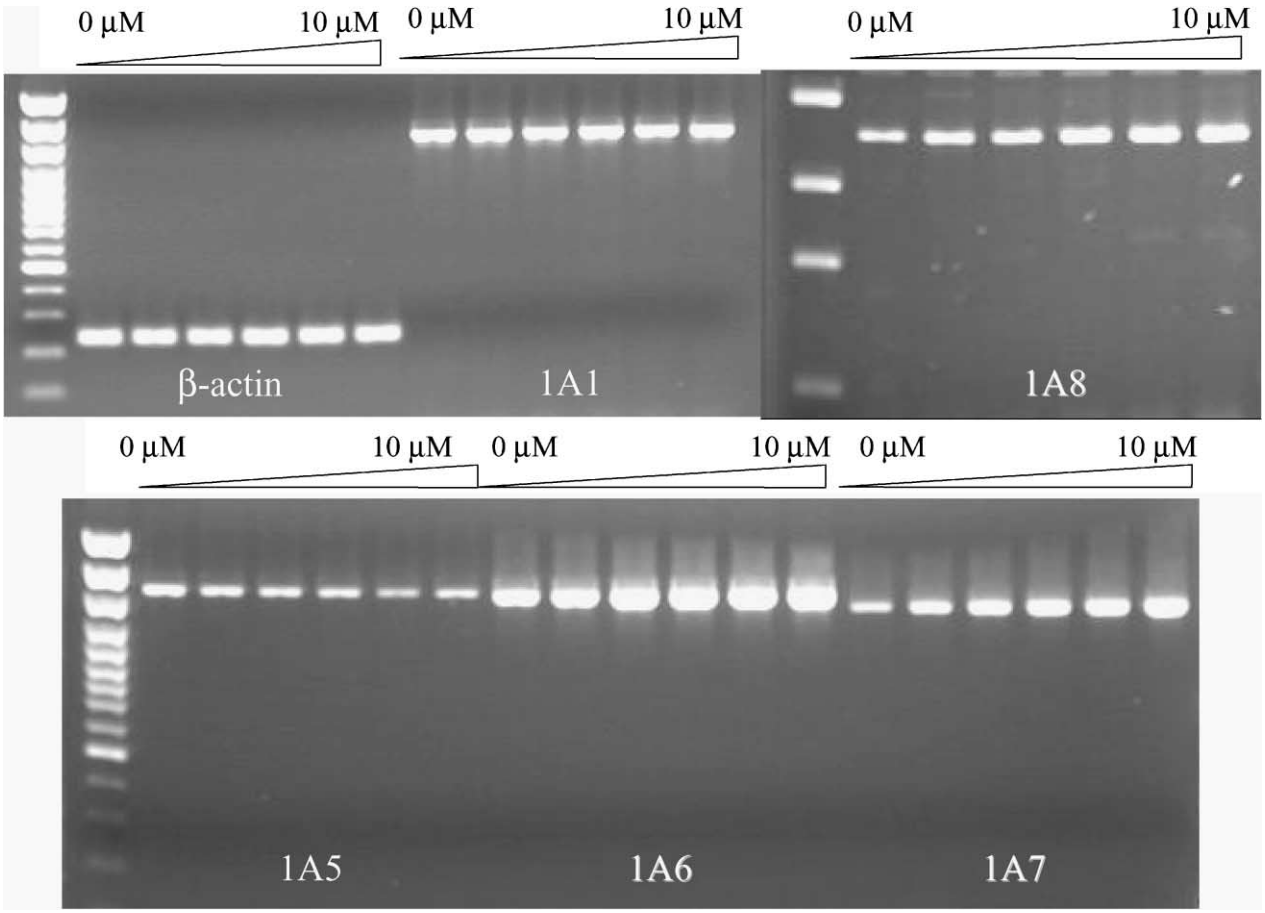

Fig. 2. UGT1A1 transcriptional activation by 3-MC in rat hepatoma H-4-II-E cells. Cells were treated with different concentrations of 3-MC: 0, 0.625, 1.25, 2.5, 5 and 10 μM. The concentration was indicated by triangle over the appropriate lane. For controls, cultures were treated with culture medium only. β-actin was used for normalization of the results.

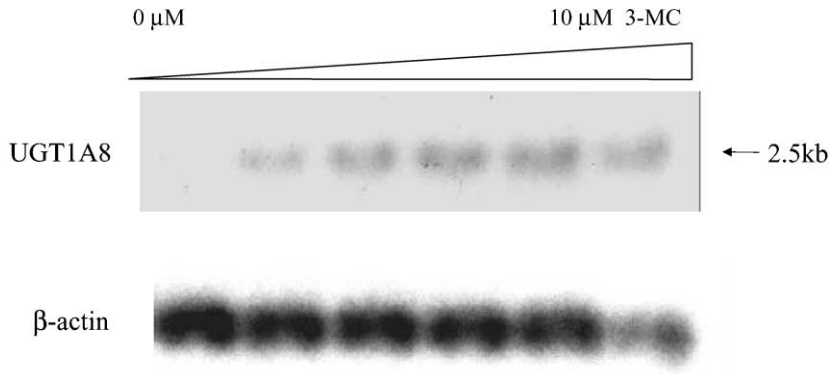

Fig. 3. Northern Blot analysis of UGT1A8 mRNA levels after treatment with different concentrations of 3-MC (0, 0.625, 1.25, 2.5, 5 and 10 μM). Hybridization of the membrane with a β-actin probe was used for normalization of the results.

to β-actin transcripts. The change reflects an increase of transcription rates by a factor of 3 (Fig. 3). These results indicated that 3-MC could regulate UGT1A8 expression in a concentration-dependent manner.

3.3. Effect of actinomycin D on UGT1A8 mRNA stability

We tested whether 3-MC stimulation is confined to induction of transcription or involves mRNA stability. Rat hepatoma cells were treated for 6 and 16 hr with 3-MC in the presence or absence of the RNA synthesis inhibitor actinomycin D (Fig. 4). In parallel, the same experiment was performed with culture medium alone as a control.

The decrease in UGT1A8 mRNA levels after inhibition of RNA synthesis were analyzed by RT-PCR as a function of time. After 16 hr treatment, 3-MC did not affect the decay of UGT1A8 mRNA levels. The data showed that 3-MC did not exert its activity on UGT1A8 mRNA levels through stabilization of mRNA.

3.4. 3-MC-induced UGT1A8 transcription is independent of de novo protein synthesis

Cycloheximide (10 μg/mL) was used as an inhibitor of protein synthesis. The results are consistent with our notion that *de novo* protein synthesis is not required. Cycloheximide treatment did not suppress UGT1A8 mRNA

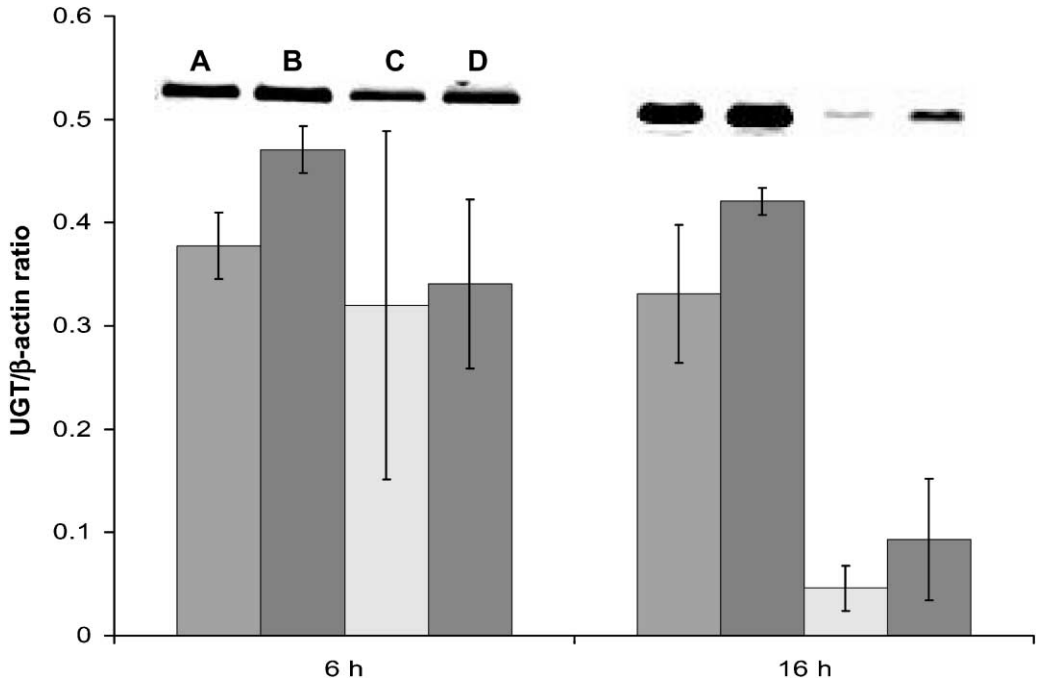

Fig. 4. Effects of actinomycin D on UGT1A8 inducibility by 3-MC after 6 and 16 hr treatment. The upper panel shows the results of RT-PCR study using UGT1A8-specific primers and the band intensity was quantified by densitometry. The values represent the mean ± SD obtained from three independent experiments. (A) control without treatment. (B) treated 10 μM 3-MC. (C) treated with 5 μg/mL actinomycin D. (D) co-treated with 10 μM 3-MC and 5 μg/mL actinomycin D.

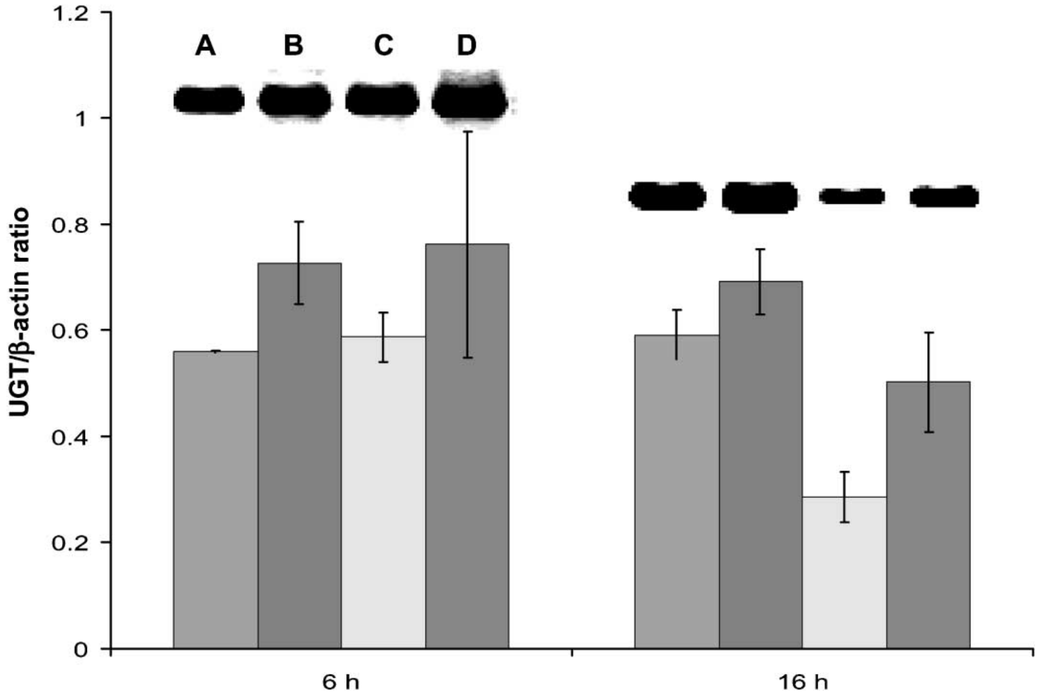

Fig. 5. Effects of cycloheximide on UGT1A8 inducibility by 3-MC after 6 and 16 hr treatment. The experiment was carried out as described in the "Method". (A) control without treatment. (B) treated 10 μM 3-MC. (C) treated with 10 μg/mL cycloheximide. (D) co-treated with 10 μM 3-MC and 10 μg/mL cycloheximide.

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                                                                              C/EBPbeta
-541  +                                                     CATGGAGTGCTCAAGGGAATGTGGAACAAAATTGTATAG   -501
      -                                                     GATATGTTAAAACAAGGTGTAAGGGAACTCGTGAGGTAC

-500  + ATCCCTTGGGGAAACCCACAGTAACTGTACCAACCTCTGTTTAGAAGATGTGACTGTCCATTGCTTTGGACATTTGAGAAGTAAAAGAGATGGCAGTCAC   -401
      - TAGGGAACCCCTTTGGGTGTCATTGACATGGTTGGAGACAAATCTTCTACACTGACAGGTAACGAAACCTGTAAACTCTTCATTTTCTCTACCGTCAGTG

-400  + ATGCCAGCAGGCCCTTGGAGTGGTCCATACTATAGTTGCCTATCTTATTTTAGCATCTCACAGCACTCCAGGAGCTTAGATGTGGAACAAGAATACTTCC   -301
      - TACGGTCGTCCGGGAACCTCACCAGGTATGATATCAACGGATAGAATAAAATCGTAGAGTGTCGTGAGGTCCTCGAATCTACACCTTGTTCTTATGAAGG

-300  + GTGGTGCCAAGACCCACAGCATTGCTCATCTGGCATTGTGGTTTCACTGTCAAAAGGGCCATGCTAAGGACCGACCCCTTAGGCCTTAGAAGGAGAGGCA   -201
      - CACCACGGTTCTGGGTGTCGTAACGAGTAGACCGTAACACCAAAGTGACAGTTTTCCCGGTACGATTCCTGGCTGGGGAATCCGGAATCTTCCTCTCCGT

-200  + GTAAATCCCTTTAGGGCACAGTGTAGTGGCCTACATTTTACTTTGAGACTAATTCTAGAAATGTAAGTTTCTTTCACACTTCGTTCCTTCCTCTTGTGGA   -101
      - CATTTAGGGAAATCCCGTGTCACATCACCGGATGTAAAATGAAACTCTGATTAAGATCTTTACATTCAAAGAAAGTGTGAAGCAAGGAAGGAGAACACCT

                            TATA box     HNF3                                                   ER
-100  + AATATTTGGACATAGAGTCAAGTATATTTCTTCATAACCATTTCTAAATTCGCGTTTCGTGATTCTGCCCAAGAAAAGGGTCACAGGCCCTTCTTATCTG     -1
      - TTATAAACCTGTATCTCAGTTCATATAAAGAAGTATTGGTAAAGATTTAAGCGCAAAGCACTAAGACGGGTTCTTTTCCCAGTGTCCGGGAAGAATAGAC

        →HNF5                                          RAR-gamma                                        XRE
   1  + AAAAACAAACATGTAGGCATCTCAATAATGGCTTGATTGTTATTGTTCTCAAAAGTCAGTCATTGTCAGGGAGTGTACTCTCTTAATGAATGTGAGTGCA    100
      - TTTTTGTTTGTACATCCGTAGAGTTATTACCGAACTAACAATAACAAGAGTTTTCAGTCAGTAACAGTCCCTCACATGAGAGAATTACTTACACTCACGT

 101  + CGCCCTCCTCTGGGGGAGGCTTTCTAACTTCCACAAGCTTTCCCCTTCCCAAATTCAAGAGAACAGAGTTCCAGCCCCTGTACTCTCCTGGTCTGCACTT    200
      - GCGGGAGGAGACCCCCTCCGAAAGATTGAAGGTGTTCGAAAGGGGAAGGGTTTAAGTTCTCTTGTCTCAAGGTCGGGGACATGAGAGGACCAGACGTGAA

 201    CTCCCATGGCTCCTTCAGGCTGTCCACCCTCCCTTCCTCTGTGTGTTTGTCTGTTCCTGGCCTCTGGCTTTGCTCAGGCAGGCAGGCTGCTGGTAGTGCC    300
              M  A  P  S  G  C  P  P  S  L  P  L  C  V  C  L  F  L  A  S  G  F  A  Q  A  G  R  L  L  V  V
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Fig. 6. Nucleotide sequence of the region flanking the UGT1A8 amino terminus coding exon. Sequences with similarity to known transcription factor binding sites are underlined. TATA, TATA-box; C/EBPβ, CAAT enhancer-binding protein beta; ER, estrogen receptor binding element; HNF3, Hepatic Nuclear Factor 3; HNF5, Hepatic Nuclear Factor 5; RAR γ, retinoic acid receptor gamma; XRE, xenobiotic responsive element; "+" indicates the DNA sequence of sense strand whereas "–" indicates the sequence of non-coding strand.

stimulation after 6 hr of treatment and showed inhibitory effects after a 16 hr treatment (Fig. 5). These results suggest that prolonged treatment with cycloheximide led to the depletion of one or more protein factors and *de novo* protein synthesis of such factors was required to maintain the induction.

The transcription initiation site was found to be located at 205 bp upstream of 1A8 coding region by 5′ RACE. With the use of DNA walking technique, 744 bp of the upstream region of UGT1A8 was successfully sequenced (Fig. 6). The potential transcription factor binding elements were analyzed by the computer software. At least seven elements were identified as the potential regulatory elements in the upstream sequence of UGT1A8. One TATA box and three common basal transcription elements including hepatic nuclear factor 3 (HNF3), hepatic nuclear factor 5 (HNF5) and CAAT enhancer binding protein beta (C/EBPβ) were recognized by the methodology. Interestingly, estrogen receptor binding element (ER) was identified near the transcription initiation site. Moreover, a higher score of retinoic acid receptor gamma (RAR γ) was found within the 5′ un-translated region (UTR) of UGT1A8 mRNA. More importantly, a conserved xenobiotic responsive element (XRE) was found to be located in the non-coding strand of 5′ UTR.

4. Discussion

In this study, we have shown that 3-MC stimulated *UGT1A8* gene expression at the transcriptional level and *de novo* protein synthesis did not seem to be required for this induction. 3-MC induction of UGT1A isozymes could enhance the activity of the corresponding enzymes in the liver. There was no change in the stability of UGT1A8 mRNA after 3-MC treatment, which suggests that post-transcriptional regulation may not contribute to the 3-MC-dependent increase in UGT1A8 activity.

3-MC was reported to regulate the expression of several other target genes at the transcriptional level [19]. *UGT1A6* and *1A7* are the typical examples of this regulation [11,14,15]. The effects of 3-MC are probably mediated through AhR that is ligand-dependent transcription factors [20,21]. These factors bind to XRE in the promoter of these genes [8,9,22,23]. Our results show that the mode of action of 3-MC on *UGT1A8* gene expression is consistent with the induction of UGT1A6 and UGT1A7 in the previous studies [14,15,22].

The biological significance of the 3-MC induction of the newly identified *UGT1A8* gene in rat hepatoma cells has not been reported. Indeed, little is known about the *UGT1A8* activity towards substrates in rats. Recent studies reported that multiple UGT isozymes are involved in the biotransformation of xenobiotics and endobiotics [24]. The protein sequence alignment of rat UGT1A7, human UGT1A9 human UGT1A10, human UGT1A7 and human UGT1A8 exhibit significant sequence identity (about 70%) to rat UGT1A8. Among these UGTs, rat UGT1A7 shows the highest identity (76%) and homology (84%) to the 1A8 isozyme. Rat UGT1A7 was found to possess a broad-spectrum glucuronidating activiy toward 12 primary (phenol) and 2 secondary (diol) benzo[α]pyrene derivatives [16]. It is believed that UGT1A8 could share some benzo[α]pyrene glucuronidating activities with rat UGT1A7 because of their high protein sequence homology. In humans, UGT1A8 showed considerable activities toward phenolic compounds, coumarins, flavonoids and anthraquinones and only minor activities toward estrogens and morphine [25–27]. In contrast to human UGT1A8, UGT1A9 in human is not only active toward exogenous compounds, particularly coumarins and flavanoids, but also possesses significant glucuronidation activities toward catecholestrogens [28]. Interestingly, human UGT1A9 appeared to be inducible by dioxin through AhR [29]. Human UGT1A9 may be the homolog of rat UGT1A7 and rat UGT1A8 as they are under similar transcriptional regulation and possess higher sequence similarity.

3-MC is known as an effective inducer for *UGT1A6* gene expression [8]. The present study has identified UGT1A8, another UGT family member, which is inducible by 3-MC. Some existing protein factors could be involved in 1A8 expression. The continuous synthesis of these protein factors are necessary for the induction. These protein factors are probably involved AhR. 3-MC is known to bind to the AhR in the cytosol and subsequently it will trigger the activation of AhR complex [30]. This is consistent with our results of cycloheximde studies in which 3-MC interacted with inactive form of AhR complex and subsequently initiated 1A8 transcription. The 3-MC–AhR complex then binds to the AhR translocator (AhNT) to form a heterodimers [31]. This complex recognizes the XRE located in the non-coding strand UGT1A8 5′ upstream region to promote gene transcription. Further incubation of cycloheximde could impair the activation because AhR may be depleted by suppression of new AhR synthesis and the turn-over of AhR.

Although, TATA box and CCAAT enhancer-binding protein beta (C/EBPβ) element were identified in the promoter region of UGT1A8, the basal transcription level remained low in the liver because no Sp1 site was identified suggesting that the constitutive expression of the UGT1A8 may be suppressed by BTE binding protein [32]. Without Sp1, some other liver-enriched factors like HNF-3, HNF-5 and C/EBPβ cannot activate the expression of UGT1A8 [33]. Besides, this UGT isozyme appears to be regulated by both estrogen and retinoic acid as their binding elements were found in the present study. However, whether these elements can function or not need further investigation as some intrinsic factors may participate in the regulation of UGT1A8 expression.

In addition, the XRE was found in the non-coding strand of the 5′ UTR with the core sequence GCGTG. Since our

results clearly demonstrate that 3-MC can induce the expression of UGT1A8 in time- and concentration-dependent manner, the discovery of XRE in the proximal upstream region of UGT1A8 reinforces the notion that 3-MC could induce 1A8 expression through the AhR. This is the first report to show that XRE in the non-coding strand could work as a normal regulatory element among the UGT isozyme studies. Similarly, the induction of UGT1A7 was believed to be mediated by AhR [15] but no XRE was identified in the coding strand of 1A7 promoter region. Only HNF1 was identified to be related to basal expression of 1A7 and XRE could act as an enhancer in the downstream region of 1A7 exon 1 to regulate the gene expression [34]. However, XRE is indeed located in −9 position near the transcription start site of UGT1A7. It appears that AhR complex can stimulate the gene expression with more than one orientation once it binds to the XRE. More work is needed to see how AhR complex interacts with the XRE.

In conclusion, the study provides information for a better understanding of the regulation of UGT1A isozymes gene expression. The effect of 3-MC is independent of *de novo* protein synthesis during the induction process.

Acknowledgments

The research was supported in part by Strategic Grant (44M3011) and a Direct Grant (2030208) from the C.U.H.K.

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