Life Sciences 258 (2020) 118140

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Expression profile of cytochrome P450s and effects of polycyclic aromatic hydrocarbons and antiepileptic drugs on CYP1 expression in MOG-G-CCM cells

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ARTICLE INFO

Keywords:
CYP1B1
CYP1A1
Astrocytes
Polycyclic aromatic hydrocarbons
Antiepileptic drugs
Aryl hydrocarbon receptor
Induction
Histone deacetylase inhibition

ABSTRACT

Aims: This study was performed to investigate the expression profile of cytochrome P450 (CYP) isoforms and effects of polycyclic aromatic hydrocarbons (PAHs) and antiepileptic drugs on CYP1 expression in human astrocytoma MOG-G-CCM cells.

Main methods: CYP1A1 and CYP1B1 expression were determined by quantitative real-time polymerase chain reaction, Western blotting, and immunocytochemistry.

Key findings: MOG-G-CCM cells expressed various CYP isoforms. Among the CYP isoforms analyzed, CYP1B1 showed the highest expression level, followed by CYP1A1. Furthermore, CYP1B1 was localized in both the endoplasmic reticulum and mitochondria. 3-Methylcholanthrene (3-MC), benz[*a*]anthracene (B[*a*]A), benzo[*a*]pyrene (B[*a*]P), and valproic acid (VPA) increased the expression of CYP1B1 and CYP1A1. The potent aryl hydrocarbon receptor antagonist GNF351 significantly suppressed the 3-MC- and VPA-mediated upregulation of CYP1B1 and CYP1A1. In addition, VPA potentiated the induction of CYP1B1 and CYP1A1 by 3-MC, B[*a*]A, and B[*a*]P, although the augmentation of CYP1A1 was more remarkable than that of CYP1B1. In contrast, other antiepileptic drugs (carbamazepine, lamotrigine, levetiracetam, phenytoin) did not affect the 3-MC-mediated upregulation of CYP1B1 and CYP1A1. VPA is known to act as a histone deacetylase (HDAC) inhibitor. Therefore, the effects of trichostatin A, a representative HDAC inhibitor, on CYP1 induction by 3-MC were examined. Trichostatin A enhanced the 3-MC-mediated upregulation of CYP1A1 but not CYP1B1.

Significance: These results partially indicated that VPA may augment the PAH-mediated induction of CYP1B1 and CYP1A1 through the activation of transcription by HDAC inhibition.

1. Introduction

Epilepsy is a chronic neurological disorder that affects more than 70 million people around the world [1]. This disorder is characterized by seizures resulting from abnormal neuronal activity in the brain and requires treatment with antiepileptic drugs (AEDs). It has been reported that the prevalence of smoking is higher in epileptic patients than in the general population in certain regions of some countries [2–4]. Smoking predisposes people to many diseases, including cardiovascular disease, cerebrovascular disease, chronic obstructive pulmonary disease, and cancers [5]. Recently, tobacco smoking has also been suggested to be a risk factor for epilepsy and epileptic seizures [6–8]. Therefore, it is clinically important to identify the effects of tobacco smoking and administration of AEDs on brain function.

Drug-metabolizing enzymes are mainly expressed in the liver and small intestine. These enzymes convert foreign chemicals into more hydrophilic compounds to rapidly excrete them from the body. Cytochrome P450 (CYP) enzymes play a major role in this biotransformation. CYP enzymes are a superfamily of heme proteins that are responsible for the oxidative metabolism of a wide variety of endogenous and exogenous compounds, including steroids, fatty acids, vitamins, drugs, procarcinogens, and xenobiotic chemicals [9]. CYP enzymes are expressed in various tissues in addition to the liver and small intestine. For example, CYP enzymes are known to be involved in the metabolism of various compounds in the brain, including centrally acting drugs, neurotoxins, neurotransmitters, and neurosteroids [10]. In general, the expression of CYP enzymes is induced by specific chemicals. In particular, the CYP1 family, which is comprised of CYP1A1, CYP1A2, and CYP1B1, is known to be induced by polycyclic aromatic hydrocarbons (PAHs) contained in tobacco smoke [11–13]. A recent

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https://doi.org/10.1016/j.lfs.2020.118140
Received 18 March 2020; Received in revised form 9 July 2020; Accepted 20 July 2020
Available online 27 July 2020

Table 1
Primers used for qPCR.

Genes	Forward primers	Reverse primers	Length (bp)	References
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG	185	[17]
CYP1A1	GTCATCTGTGCCATTTGCTTTG	CAACCACCTCCCCGAAATTATT	97	[18]
CYP1A2	TGTTCAAGCACAGCAAGAAGG	TGCTCCAAAGACGTCATTGAC	92	[18]
CYP1B1	CTCCTCCTCTTCACCAGGTATCCT	CATACAAGGCAGACGGTCCCT	90	[19]
CYP2A6	GAGTTCCTGTCACTGTTGCG	GTCCTGGCAGGTGTTTCATC	109	[20]
CYP2B6	CCCCAAGGACACAGAAGTATTTC	GATTGAAGGCGTCTGGTTTTTC	83	[18]
CYP2C8	CTCGGGACTTTATGGATTGC	CAGTGCCAACCAAGTTTTCA	93	[20]
CYP2C9	TGCTTCCTGATGAAAATGGA	TCTCTGTCCCAGCTCCAAAC	106	[20]
CYP2C19	TTGCTTCCTGATCAAAATGG	GTCTCTGTCCCAGCTCCAAG	108	[20]
CYP2D6	TGCATGTGGACTTCCAGAAC	CAGCCCATTGAGCACGAC	113	[20]
CYP2E1	GACCACCAGCACAACTCTGA	CCCAATCACCCTGTCAATTT	97	[20]
CYP2J2	GGCAACTTCTTCCTTGTGGA	TGTCACCAAGCTCCAAGCTA	97	[20]
CYP3A4	CCTTACACATACACACCCTTTGGAAG	TGGTTGAAGAAGTCCTCCTAAGCT	171	[21]
CYP3A5	CCTTACATATACACACCCTTTGGAAC	GTTGAAGAAGTCCTTGCGTGTC	169	[21]
CYP3A7	GGGAAATGCTTTGTCCTTCC	AGCCAGCATAGGCTGTTGAC	109	[20]
CYP4A11	CTCAAAGCCCTCCAGCAGT	ACCCATTTCTGAATCCGTTG	104	[20]
CYP4A22	ATAGGCAGTGGCTGCTCAAA	GAATCCGTTGTAGCTCCTGG	108	[20]
CYP4F2	GGCAGGAGGTGCAAGAACT	TCATGCACATGGTCAGGAAG	93	[20]
CYP4F3	CAAGAGCTTCTGAAGGACCG	CCTCAGGCTCTCCTTAATGC	96	[20]
CYP4F8	TGAAGGACCGTGAGCCTAAA	GAATGTAGGGATTGGGGGAT	110	[20]
CYP4F11	CAAGAGCTTCTGAAGGACCG	GCAGGCTCTCCTTAATGCAC	94	[20]
CYP4F12	CAAGAGCTTCTGAAGGACCG	GGGATGTAACCTCAGGCTCTC	105	[20]
CYP46A1	TCTGTCCCAGGCAGTGAAAC	AGCTGCTTCCTCTTCCCTG	90	[20]

study showed that the expression levels of CYP1A1 and CYP1B1 in the prefrontal cortex and/or amygdala tended to be higher in smokers than in non-smokers [14]. These findings suggest that PAHs contained in tobacco smoke may be involved in the regulation of cerebral *CYP1* gene expression. As CYP1 enzymes are capable of hydroxylating the neurosteroid 17β-estradiol [15,16], brain CYP1 enzymes have been suggested to play a role in maintaining the function of neurosteroids. If hyperupregulation of *CYP1* gene expression by interaction between tobacco smoke components and drugs occurs in the human brain, biological responses to neurosteroids may be altered and hence affect brain function. However, the effects of PAHs on cerebral *CYP1* gene expression have not been extensively investigated. In particular, there have been no reports regarding the role of the interaction of PAHs with AEDs in the regulation of cerebral *CYP1* gene expression.

In this study, we investigated the expression profile of CYP isoforms and effects of PAHs and AEDs on CYP1 expression in the human astrocytoma cell line MOG-G-CCM.

2. Materials and methods

2.1. Reagents and antibodies

3-Methylcholanthrene (3-MC) was purchased from Nacalai Tesque (Kyoto, Japan). Aryl hydrocarbon receptor (AhR) antagonist III (GNF351) was obtained from Merck KGaA (Darmstadt, Germany). Other reagents and antibodies were obtained from the following sources: acenaphthene, acenaphthylene, benz[*a*]anthracene (B[*a*]A), benzo[*a*]pyrene (B[*a*]P), fluoranthene, fluorene, 1-methylnaphthalene, naphthalene, phenanthrene, pyrene, carbamazepine (CBZ), and 16% (w/v) methanol-free paraformaldehyde solution from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); anthracene, 2-methylnaphthalene, lamotrigine (LTG), 5,5-diphenylhydantoin sodium salt (phenytoin; PHT), valproic acid sodium salt (VPA), and an anti-actin (20 − 33) antibody produced in rabbit from Sigma-Aldrich (St. Louis, MO, USA); levetiracetam (LEV) from LKT Laboratories (St. Paul, MN, USA); trichostatin A (TSA) from Cayman Chemical (Ann Arbor, MI, USA); goat anti-rabbit IgG-HRP (catalog# sc-2004) and rabbit polyclonal antibodies against human CYP1B1 (H-105) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); normal goat serum blocking solution from Vector Laboratories (Burlingame, CA, USA); mouse monoclonal antibodies against human cytochrome *c* oxidase subunit IV (COX IV) (mAbcam33985) and human calnexin (AF18) from Abcam (Cambridge, MA, USA); Alexa Fluor® 488-conjugated goat anti-mouse IgG (H + L) F(ab′)$_2$ fragment from Cell Signaling Technology (Beverly, MA, USA); Alexa Fluor® 546-conjugated goat anti-rabbit IgG (H + L) highly cross-absorbed antibody from Molecular Probes (Eugene, OR, USA); Hoechst 33258 from Setareh Biotech (Eugene, OR, USA); baculovirus-infected insect cell microsomes expressing human CYP1B1 with human NADPH-CYP reductase (CYP1B1 Supersomes™) from Corning Incorporated (Woburn, MA, USA). All other chemicals and reagents were of the highest quality commercially available.

2.2. Cell culture and drug treatments

The human astrocytoma cell line MOG-G-CCM was obtained from the European Collection of Authenticated Cell Cultures (Salisbury, UK). MOG-G-CCM cells were maintained in Dulbecco's modified Eagle's medium/nutrient mixture F-12 Ham (Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (BioWest, Nuaillé, France) and penicillin/streptomycin (Gibco, Grand Island, NY, USA) in a humidified atmosphere containing 5% CO_2 at 37 °C. The culture medium was replaced with fresh medium every 2–3 days. Cells were seeded into 6-well plates (1×10^5 cells/well) and cultured for 24 h. Cells were treated with PAHs, AEDs, and/or TSA in serum-free medium for up to 48 h. Antagonism experiments were carried out as described below. Cells were treated with GNF351 in the presence or absence of 3-MC and VPA for 12 h. With the exception of VPA that was dissolved in serum-free medium, all test chemicals were prepared in dimethylsulfoxide. Controls were prepared with equivalent volumes (0–0.525%) of dimethylsulfoxide only, which did not significantly influence cell viability and expression levels of target genes at the final volume used.

2.3. RNA analysis

Total RNA was extracted from MOG-G-CCM cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription reactions were conducted with a High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time polymerase chain reaction (qPCR) was performed using an ABI 7300 real-time PCR system (Applied Biosystems) with a KAPA SYBR® Fast qPCR Kit (Kapa Biosystems, Boston, MA, USA) according to the manufacturer's instructions. The primers used are

summarized in Table 1. Relative expression levels of target genes were quantified by the comparative Ct method ($2^{-\Delta\Delta Ct}$), and samples were normalized relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.4. Western blotting analysis

Whole-cell lysates were prepared from MOG-G-CCM cells by scraping in 100 μl of lysis buffer (150 mM sodium chloride, 50 mM Tris-HCl, pH 8.0, 0.1% sodium dodecyl sulfate, 5 mM ethylenediamine tetraacetic acid, 1% sodium deoxycholate, 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail (cOmplete™ Mini, Roche Diagnostics, Meylan, France), and 2.5 g/l sodium azide). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed using 10% polyacrylamide gels as described previously [22]. Total cellular protein (15 μg protein) and CYP1B1 Supersomes™ (10 fmol P450) were separated and transferred onto polyvinylidene fluoride membranes. Membranes were blocked at room temperature for 1 h with 5% nonfat milk in phosphate buffered saline (PBS) containing 0.1% Tween 20 (PBS/T) and then incubated overnight at 4 °C with primary antibodies against CYP1B1 (1:1000 dilution) or actin (1:2000 dilution) in PBS/T. Membranes were incubated at room temperature for 1 h with goat anti-rabbit IgG-HRP (1:2000 dilution for CYP1B1, 1:5000 dilution for actin) as a secondary antibody in PBS/T. Conjugated horseradish peroxidase was detected using Immobilon™ Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA), and bands were scanned with a ChemiDoc XRS Plus system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.5. Immunocytochemistry

MOG-G-CCM cells were seeded into 24-well plates (2×10^4 cells/well) and cultured for 24 h. Cells were fixed with 4% paraformaldehyde in PBS at room temperature for 20 min and then incubated in blocking buffer (0.3% Triton X-100 and 5% normal goat serum in PBS) at room temperature for 1 h. Fixed cells were incubated overnight at 4 °C with primary antibodies against CYP1B1, COX IV, or calnexin (1:200 dilution) in blocking buffer. Fixed cells were incubated at room temperature for 1 h with Alexa Fluor® 488-conjugated goat anti-mouse IgG or Alexa Fluor® 546-conjugated goat anti-rabbit IgG (1:1000 dilution) as secondary antibodies in blocking buffer. After counterstaining of nuclei with Hoechst 33258 (5 μg/ml), fluorescence images were obtained using a Biozero BZ-8100 (Keyence, Osaka, Japan) at 20× magnification.

2.6. Statistical analysis

Data are expressed as the mean ± standard deviation (S.D.) of triplicate determinations. The results were analyzed by one-way analysis of variance followed by Dunnett's or Bonferroni's multiple comparison test with statistical significance set at $p < 0.05$. All statistical analyses were performed with GraphPad Prism 5.02 software (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Expression profile of human CYP isoforms in MOG-G-CCM cells

The relative expression levels of human CYP isoforms in MOG-G-CCM cells were measured by qPCR. CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP4F2, CYP4F3, CYP4F11, CYP4F12, and CYP46A1 expression were detected in MOG-G-CCM cells (Table 2). Among the CYP isoforms analyzed, CYP1B1 showed the highest expression level followed by CYP1A1.

Table 2
Relative expression of individual CYP mRNAs in MOG-G-CCM cells.

Isoforms	Relative mRNA levels
CYP1A1	23.44 ± 4.55
CYP1A2	0.11 ± 0.12
CYP1B1	5677.31 ± 529.38
CYP2A6	N.D.
CYP2B6	3.50 ± 0.91
CYP2C8	7.43 ± 2.37
CYP2C9	N.D.
CYP2C19	N.D.
CYP2D6	N.D.
CYP2E1	1.19 ± 0.67
CYP2J2	4.55 ± 0.69
CYP3A4	1.03 ± 0.29
CYP3A5	4.76 ± 0.99
CYP3A7	N.D.
CYP4A11	N.D.
CYP4A22	N.D.
CYP4F2	2.57 ± 2.20
CYP4F3	1.08 ± 0.51
CYP4F8	N.D.
CYP4F11	0.68 ± 0.68
CYP4F12	0.53 ± 0.34
CYP46A1	1.63 ± 0.58

Values are expressed as fold change (mean ± S.D. of triplicate determinations) in gene expression relative to CYP3A4.
N.D.; not detectable (relative mRNA levels < 0.01).

3.2. Intracellular localization of human CYP1B1 in MOG-G-CCM cells

In general, hepatic CYP enzymes involved in drug metabolism are predominantly localized in the endoplasmic reticulum (ER; also termed microsomes), although a small portion of these enzymes are also found in mitochondria [23]. On the other hand, drug-metabolizing brain CYP enzymes have been reported to be localized in both the ER and mitochondria [24,25]. Therefore, to determine the subcellular distribution of CYP1B1 most highly expressed in MOG-G-CCM cells, we carried out immunocytochemical analysis. CYP1B1 was widely distributed in the cells; some was co-localized with the ER marker calnexin [26] (Fig. 1A), and some was co-localized with the mitochondrial marker COX IV [27] (Fig. 1B).

3.3. Inducibility of human CYP1 expression by 3-MC and tobacco smoke containing PAHs in MOG-G-CCM cells

The inducibility of CYP1B1 and CYP1A1 by 3-MC, a representative PAH inducing CYP1 expression [28], was evaluated in MOG-G-CCM cells. 3-MC increased the expression of CYP1B1 mRNA in a concentration-dependent manner, reaching the maximum level at 0.3 μM (Fig. 2A). The expression reached a maximum level at 24 h after 3-MC treatment (Fig. 2B). Treatment with 3-MC also upregulated the expression of CYP1B1 protein in a concentration-dependent manner (Fig. 2C). Under the same conditions as CYP1B1 mRNA, 3-MC showed concentration-dependent induction of CYP1A1 mRNA (Supplementary material 1A). The expression level reached a maximum at 12 h after treatment with 3-MC (Supplementary material 1B).

The activation of *CYP1* gene transcription by 3-MC is mediated by the AhR, which is a transcription factor of the basic-helix-loop-helix/Per-Arnt-Sim family [29]. To determine whether the AhR was involved in the induction of CYP1B1 and CYP1A1 by 3-MC in MOG-G-CCM cells, the effects of an AhR antagonist on 3-MC-mediated upregulation were examined. We used GNF351 as an AhR antagonist. This compound is a high-affinity AhR ligand without agonist activity and has the capacity to repress both xenobiotic response element-dependent and -independent AhR functions [30]. GNF351 significantly suppressed the

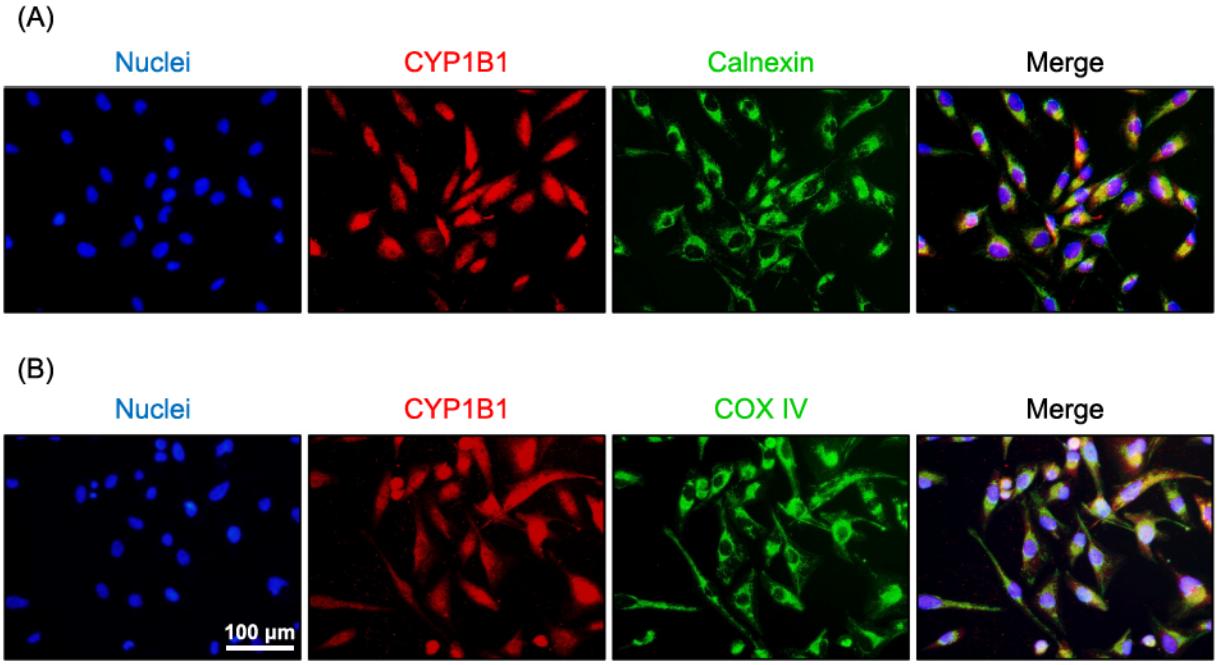

Fig. 1. Immunocytochemical detection of CYP1B1, calnexin, and COX IV in MOG-G-CCM cells. MOG-G-CCM cells were incubated with antibodies against CYP1B1 (red) and calnexin (ER marker, green) (A) or antibodies against CYP1B1 (red) and COX IV (mitochondrial marker, green) (B) for double-labeled immunofluorescence. The cell nuclei were stained with Hoechst 33258 (blue). The merged images show CYP1B1 (yellow) localized in the ER (A) and mitochondria (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

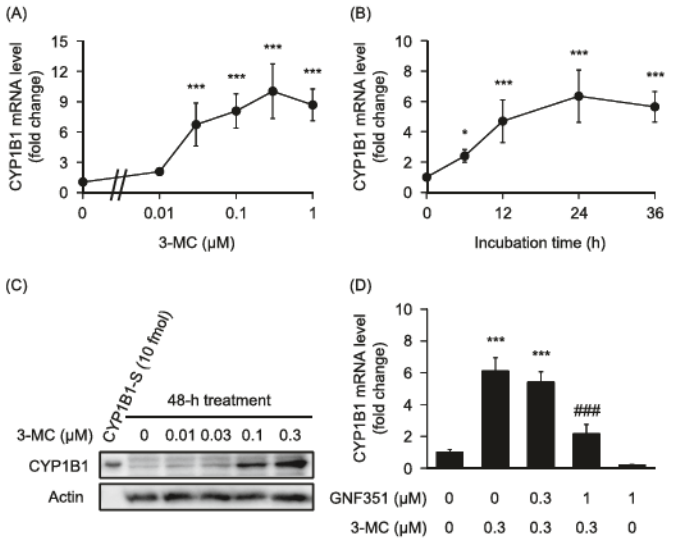

Fig. 2. Effects of 3-MC on expression of CYP1B1 in MOG-G-CCM cells. (A) MOG-G-CCM cells were treated with 3-MC for 24 h. Expression level of CYP1B1 mRNA was determined by qPCR. Data are expressed as the mean ± S.D. of triplicate determinations. ***$p < 0.001$ vs. control (Dunnett's test). (B) MOG-G-CCM cells were treated with 3-MC (0.3 μM) for the indicated periods. Expression level of CYP1B1 mRNA was determined by qPCR. Data are expressed as the mean ± S.D. of triplicate determinations. *$p < 0.05$, ***$p < 0.001$ vs. control (Dunnett's test). (C) MOG-G-CCM cells were treated with 3-MC for 48 h. CYP1B1 and actin proteins were detected by Western blotting. CYP1B1-S; CYP1B1 Supersomes™. (D) MOG-G-CCM cells were treated with GNF351 in the presence or absence of 3-MC for 12 h. Expression level of CYP1B1 mRNA was determined by qPCR. Data are expressed as the mean ± S.D. of triplicate determinations. ***$p < 0.001$ vs. control, $^{###}p < 0.001$ vs. 3-MC alone (Bonferroni's test).

induction of CYP1B1 mRNA by 3-MC (Fig. 2D). Treatment with GNF351 also significantly reduced the expression level of CYP1A1 mRNA induced by 3-MC (Supplementary material 1C).

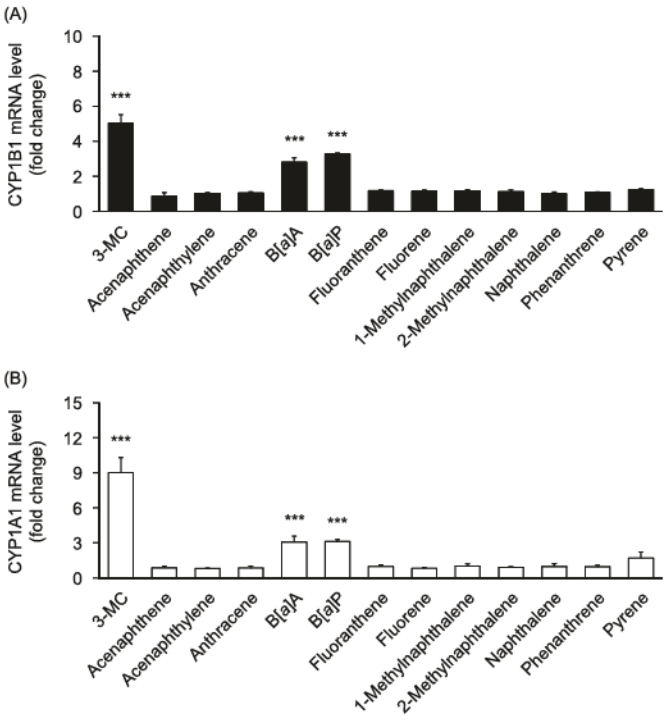

Fig. 3. Effects of tobacco smoke containing PAHs on CYP1 expression in MOG-G-CCM cells. MOG-G-CCM cells were treated with 3-MC or major PAHs contained in tobacco smoke (0.3 μM) for 24 h. Expression levels of CYP1B1 (A) and CYP1A1 (B) mRNAs were determined by qPCR. Data are expressed as the mean ± S.D. of triplicate determinations (n = 4 per an experiment). ***$p < 0.001$ vs. control (Dunnett's test).

The inducibilities of CYP1B1 and CYP1A1 by major twelve PAHs contained in tobacco smoke [31] were investigated in MOG-G-CCM cells. Among the PAHs tested, B[*a*]A and B[*a*]P significantly upregulated the expression of CYP1B1 and CYP1A1 mRNAs (Fig. 3).

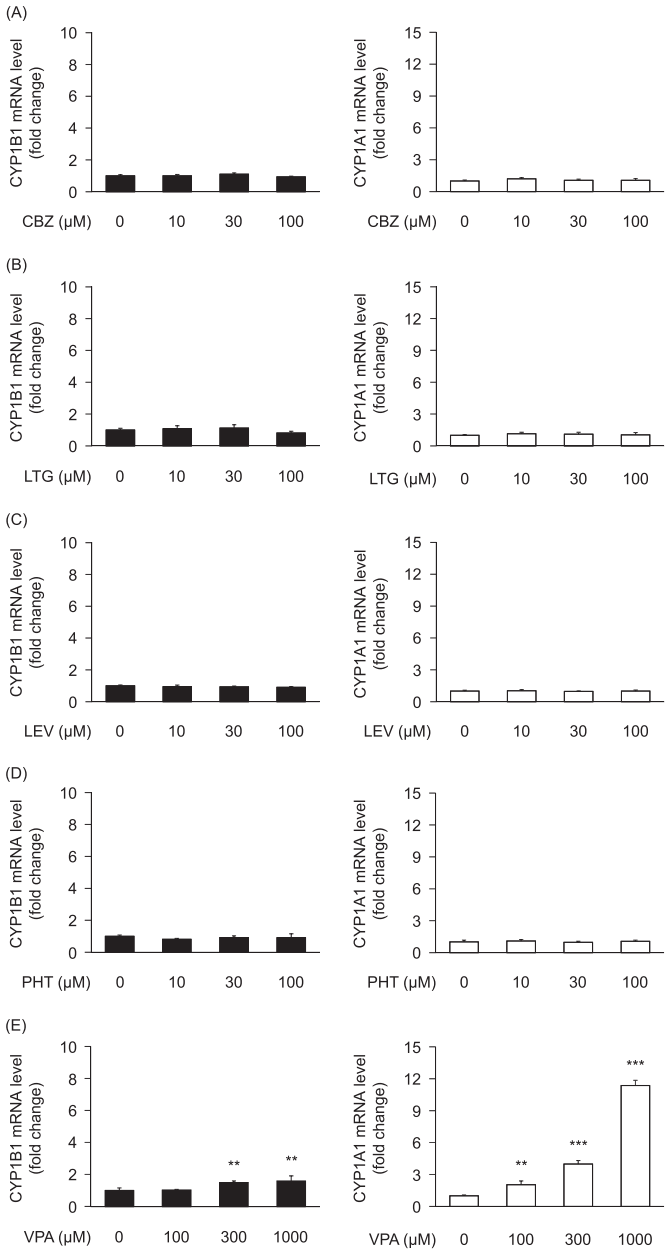

Fig. 4. Effects of AEDs on CYP1 expression in MOG-G-CCM cells. MOG-G-CCM cells were treated with CBZ (A), LTG (B), LEV (C), PHT (D), and VPA (E) for 12 h. Expression levels of CYP1B1 and CYP1A1 mRNAs were determined by qPCR. Data are expressed as the mean ± S.D. of triplicate determinations (n = 3–4 per an experiment). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (Dunnett's test).

3.4. Effects of AEDs on human CYP1 expression in MOG-G-CCM cells

The inducibilities of CYP1B1 and CYP1A1 by AEDs were evaluated in MOG-G-CCM cells. We set the concentrations of CBZ, LTG, LEV, PHT, and VPA based on these clinically relevant concentrations [32]. CBZ, LTG, LEV, and PHT did not influence the expression of CYP1B1 and CYP1A1 mRNAs under the current conditions (Fig. 4A–D, Supplementary material 2A–D). On the other hand, treatment with VPA for 12 h significantly induced the expression of CYP1B1 and CYP1A1 (Fig. 4E), whereas treatment for 24 h showed an increase in CYP1A1 mRNA but not CYP1B1 mRNA level (Supplementary material 2E). To determine the involvement of AhR in VPA-mediated induction of CYP1B1 and CYP1A1, the effects of an AhR antagonist on their upregulation were investigated. Treatment with GNF351 significantly repressed the

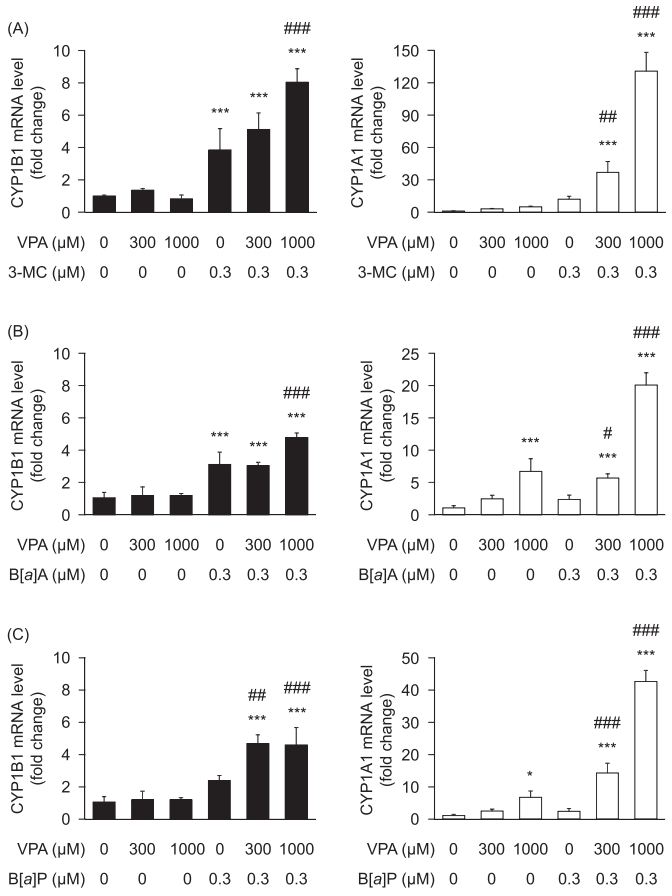

Fig. 5. Effects of VPA on CYP1 induction by PAHs in MOG-G-CCM cells. MOG-G-CCM cells were treated with VPA in the presence or absence of 3-MC (A), B[*a*]A (B), and B[*a*]P (C) for 24 h. Expression levels of CYP1B1 and CYP1A1 mRNAs were determined by qPCR. Data are expressed as the mean ± S.D. of triplicate determinations (n = 3–4 per an experiment). $^{*}p < 0.05$, $^{***}p < 0.001$ vs. control, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. PAH alone (Bonferroni's test).

induction of CYP1B1 and CYP1A1 mRNAs by VPA (Supplementary material 3).

Next, the effects of AEDs on CYP1 induction by PAHs were examined. Co-treatment with CBZ, LTG, LEV, and PHT for 24 h did not affect 3-MC-mediated induction of CYP1B1 and CYP1A1 mRNAs (Supplementary material 4). In contrast, VPA significantly enhanced the upregulation of CYP1B1 and CYP1A1 mRNAs by 3-MC (Fig. 5A). VPA also significantly increased the expression levels of CYP1B1 and CYP1A1 mRNAs induced by B[*a*]A and B[*a*]P (Fig. 5B and C). These effects were more remarkable in induction of CYP1A1 than CYP1B1.

3.5. Effects of TSA on human CYP1 induction by 3-MC in MOG-G-CCM cells

VPA is known to act as an inhibitor of histone deacetylases (HDACs) [33,34]. HDACs regulate the acetylation status of histones. HDAC inhibition promotes histone acetylation, resulting in the activation of gene expression [35]. A mechanism by which VPA augmented PAH-mediated upregulation of *CYP1* gene expression was considered to be due to inhibition of HDACs by VPA. To clarify whether CYP1 induction by 3-MC was potentiated by HDAC inhibition, the effects of an HDAC inhibitor on 3-MC-mediated induction were examined. TSA, a representative HDAC inhibitor [36], significantly enhanced 3-MC-mediated upregulation of CYP1A1 mRNA, whereas this inhibitor did not potentiate that of CYP1B1 mRNA (Fig. 6).

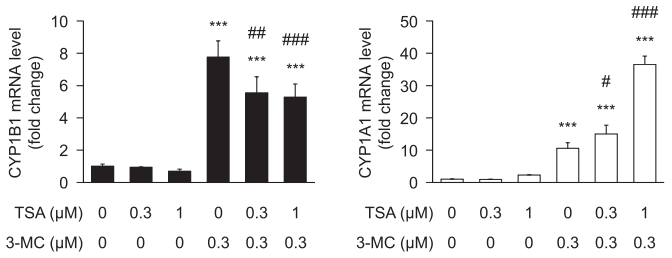

Fig. 6. Effects of TSA on CYP1 induction by 3-MC in MOG-G-CCM cells. MOG-G-CCM cells were treated with TSA in the presence or absence of 3-MC for 24 h. Expression levels of CYP1B1 and CYP1A1 mRNAs were determined by qPCR. Data are expressed as the mean ± S.D. of triplicate determinations (n = 4 per an experiment). $^{***}p < 0.001$ vs. control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. 3-MC alone (Bonferroni's test).

4. Discussion

In the present study, we demonstrated that a variety of CYP isoforms, including CYP1B1 and CYP1A1, are expressed in human astrocytoma MOG-G-CCM cells. It has been reported that CYP1B1 and CYP1A1 are expressed in MOG-G-CCM cells [37–39]. To our knowledge, however, this is the first study to show the expression of many CYP isoforms other than CYP1B1 and CYP1A1 in this cell line. Furthermore, our results showed that CYP1B1 had the highest expression level among the CYP isoforms examined. To date, the expression of CYP1B1 has been found in astrocytes and neurons from various regions of the normal human brain (e.g., frontal cortex, hippocampus, cerebellum, etc.) [25]. Interestingly, the cellular distribution of CYP1B1 varies greatly among brain regions. The expression of CYP1B1 in glial cells, such as astrocytes and microglia, is detected in the frontal cortex, hippocampus, and cerebellum [25]. On the other hand, the neuronal expression of CYP1B1 is not found in the frontal cortex, although this expression is restricted to the granular layer of the hippocampus and glomeruli of the cerebellum [25]. These differences in cellular distribution are considered to be due to differences in cellular composition, cell density, and function of glial cells and neurons in individual brain regions. In addition to the normal brain, CYP1B1 is highly expressed in various brain tumors (glioblastoma, astrocytoma, neuroblastoma, etc.) [40–44]. In glial tumors, increased expression of CYP1B1 has been shown to be associated with decreased patient survival [42]. These findings suggest that CYP1B1 may play important roles not only in normal brain function but also in the pathogenesis and malignancy of brain tumors.

Although there have been several reports on the expression of CYP1B1 in MOG-G-CCM cells as described above, its subcellular localization was unclear. In this study, we demonstrated the possibility that CYP1B1 may be localized not only in the ER but also in the mitochondria of MOG-G-CCM cells. The mitochondrial localization of CYP1B1 has also been observed in some regions of the normal human brain [25] and the human neuroblastoma cell line SH-SY5Y [44]. Mitochondrial targeting of CYP1B1 has been reported to involve proteolytic processing of the N-terminus of the nascent protein by a cytosolic endoprotease [45]. Further studies are needed to elucidate the role of CYP1B1 localized in the brain mitochondria.

There have been a few previous studies on the induction of CYP1B1 and/or CYP1A1 by PAHs, such as 7,12-dimethylbenz[*a*]anthracene and 3-MC, in MOG-G-CCM cells [38,39]. Our study confirmed that 3-MC and two tobacco smoke containing PAHs, B[*a*]A and B[*a*]P, significantly induced the expression of CYP1B1 and CYP1A1 in MOG-G-CCM cells. However, McFadyen et al. [39] reported that a 3-MC-mediated increase in CYP1B1 mRNA level was not observed in this cell line. This discrepancy may be explained by the differences in concentrations of 3-MC tested; they used 3-MC at a very high concentration (25 μM), whereas we applied this compound at a maximum of 1 μM. In this study, the induction of CYP1B1 by 3-MC reached a maximum level at 0.3 μM and plateaued at 1 μM. These findings suggested that a high concentration of 3-MC (25 μM) may lack the ability to induce the expression of CYP1B1 in MOG-G-CCM cells. Moreover, our study using AhR antagonist showed the involvement of AhR in the induction of CYP1B1 and CYP1A1 by 3-MC in MOG-G-CCM cells. B[*a*]A and B[*a*]P, like 3-MC, are also AhR ligands [46,47]. Tobacco smoke contains a variety of PAHs other than the compounds tested in this study [31]. Many PAHs including benzo[*k*]fluoranthene and chrysene, as well as B[*a*]A and B[*a*]P, have an ability to induce CYP1 expression via AhR signaling [13,48]. Our results indicated that MOG-G-CCM cells possess inducibilities of CYP1B1 and CYP1A1 by PAHs.

The results of expression analysis with AEDs revealed that only VPA induced the expression of CYP1B1 and CYP1A1 in MOG-G-CCM cells. In addition, the VPA-mediated induction of CYP1B1 and CYP1A1 was repressed by an AhR antagonist. These results suggest that VPA may induce the expression of CYP1B1 and CYP1A1 through the activation of AhR signaling. There are at least two mechanisms underlying the transcriptional activation of the *CYP1* genes through the AhR; a ligand-dependent AhR activation by AhR agonists, such as 3-MC, and ligand-independent AhR activation by certain chemicals, such as omeprazole [29]. At present, however, it remains unclear whether VPA acts as an AhR ligand. The upregulation of CYP1A1 by VPA has been also observed in the human cerebral microvascular endothelial cell line hCMEC/D3 and human neuroblastoma cell lines UKF-NB-3 and UKF-NB-4 [49,50]. Furthermore, several other HDAC inhibitors have been shown to possess an ability to induce the expression of CYP1B1 and/or CYP1A1 in some cell lines [49–52]. A recent study suggests that the induction of CYP1A1 by HDAC inhibitors is caused by increasing histone acetylation and the amount of AhR bound to the xenobiotic response element at the *CYP1A1* gene promoter [49]. These findings suggest that the HDAC inhibition by VPA also may play an important role in the VPA-mediated upregulation of CYP1B1 and CYP1A1 in MOG-G-CCM cells. In contrast to VPA, CBZ, LTG, LEV, and PHT did not cause the induction of CYP1B1 and CYP1A1 in this cell line under the current conditions. Of these AEDs, CBZ and PHT are known to induce the expression of CYP2C and CYP3A [53]. Moreover, CBZ is also capable of inducing CYP1A2 in the liver [54,55]. However, there are no reports regarding the induction of CYP1B1 and CYP1A1 by CBZ, LTG, LEV, and PHT. At present, roles of these AEDs in the regulation of *CYP1B1* and *CYP1A1* gene expression remain to be elucidated.

The present study indicated that VPA alone among the AEDs tested enhanced the induction of CYP1B1 and CYP1A1 by PAHs in MOG-G-CCM cells. This is the first report showing VPA-mediated potentiation of CYP1B1 induction by PAHs, although VPA has been reported to augment PAH-mediated CYP1A1 induction [56]. VPA has an ability to inhibit HDAC activity [33,34,49]. HDAC inhibition facilitates the acetylation of histone, resulting in the transcriptional activation of genes [35]. Thus, we considered a possibility that VPA-mediated enhancement of CYP1 induction by PAHs was caused by HDAC inhibition. To confirm this hypothesis, we examined the effects of TSA, a representative HDAC inhibitor, on 3-MC-mediated induction. The results showed that TSA augmented the induction of CYP1A1 by 3-MC. It has been shown that several HDAC inhibitors, including VPA and TSA, potentiated PAH-mediated CYP1A1 induction in other cell lines [49,52,56,57]. These results suggest that VPA may promote PAH-mediated transcriptional activation of the *CYP1A1* gene by increasing histone acetylation in MOG-G-CCM cells. On the other hand, TSA failed to potentiate 3-MC-mediated induction of CYP1B1 in this cell line. A similar result has been also observed in the human breast cancer cell line MCF-7 co-treated with B[*a*]P and TSA [51]. The reason why TSA was not responsive to PAH-mediated CYP1B1 induction is unclear. In contrast to TSA, it has been reported that other HDAC inhibitors, butyric acid, panobinostat, and vorinostat, increased the expression of CYP1B1 induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent AhR agonist, in the human colonic adenocarcinoma cell line Caco-2 [52].

These findings may partially explain the potential involvement of histone acetylation in VPA-mediated augmentation of CYP1B1 induction by PAHs in MOG-G-CCM cells.

In the present study, we identified part of the mechanism of high CYP1B1 and CYP1A1 expression in the brains of smokers reported previously by Toselli et al. [14]. Furthermore, among the AEDs tested, we demonstrated that only VPA augmented PAH-mediated CYP1 induction. CYP1B1 and CYP1A1 are known to be involved in the metabolism of estrogen, one of the neurosteroids [15,58]. It is suggested that neurosteroids play important roles in neural circuit building, brain repair, memory, learning, and cognitive functioning [59]. If excessive response of *CYP1* genes to tobacco smoking is induced in the brains of smokers receiving VPA, this response may enhance estradiol metabolism in the brain and hence affect the maintenance of normal brain function. In addition, it has been reported that neurosteroids, especially allopregnanolone, have anticonvulsant effects [60]. These findings suggest that dysfunction of neurosteroid biosynthesis caused by smoking and VPA administration may lead to the onset of epileptic seizures. Further studies are needed to clarify the physiological functions of neurosteroids and the roles of cerebral CYP1 enzymes in epilepsy.

5. Conclusion

We demonstrated that human astrocytoma MOG-G-CCM cells expressed various CYP isoforms; CYP1B1 was most highly expressed, followed by CYP1A1. 3-MC, B[*a*]A, B[*a*]P, and VPA effectively induced the expression of CYP1B1 and CYP1A1 in this cell line. In addition, VPA significantly potentiated the induction of CYP1B1 and CYP1A1 by these PAHs. Our results partially suggest that the VPA-mediated augmentation of CYP1 induction by PAHs is mediated by HDAC inhibition by VPA. Our study provided useful basic information regarding the effects of tobacco smoking and AEDs on brain function.

Author contributions

Participated in conception and design of the study: Shusuke Ozawa, Satoshi Yamaori, Shigeru Ohmori

Conducted experiments: Shusuke Ozawa, Satoshi Yamaori, Kaori Aikawa, Shinobu Kamijo

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Wrote or contributed to the writing of the manuscript: Shusuke Ozawa, Satoshi Yamaori, Shigeru Ohmori

Acknowledgements

None.

Funding

This work was supported in part by Grants-in-Aid for Encouragement of Scientists from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Nos. 25926005, 26926003, 15H00481, 16H00486, and 17H00475).

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2020.118140.

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