Toxicology Letters 208 (2012) 51–61

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

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

Transcriptional and posttranslational inhibition of dioxin-mediated induction of CYP1A1 by harmine and harmol

Mohamed A.M. El Gendy[a], Anatoly A. Soshilov[b], Michael S. Denison[b], Ayman O.S. El-Kadi[a,*]

[a] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8
[b] Department of Environmental Toxicology, University of California, Davis, CA 95616, United States

ARTICLE INFO

Article history:
Received 2 June 2011
Received in revised form
13 September 2011
Accepted 14 September 2011
Available online 6 October 2011

Keywords:
Aryl hydrocarbon receptor
Carcinogenesis
CYP 1A1
Harmine
Harmol

ABSTRACT

Dioxins are widespread environmental contaminants that induce the carcinogen-activating enzyme, cytochrome P450 1A1 (CYP1A1) through an aryl hydrocarbon receptor (AhR)-dependent mechanism. We previously demonstrated that harmine inhibits the dioxin-mediated induction of Cyp1a1 activity in murine hepatoma cells. Therefore, the aim of this study is to determine the effect of harmine and its main metabolite, harmol, on the dioxin-mediated induction of CYP1A1 in human HepG2 and murine Hepa 1c1c7 hepatoma cells. Our results showed that harmine and harmol significantly inhibited the dioxin-mediated induction of CYP1A1 at mRNA, protein, and activity levels in a concentration-dependent manner in human and murine hepatoma cells. Moreover, harmine and harmol inhibited the AhR-dependent luciferase activity and the activation and transformation of AhR using the electrophoretic mobility shift assay. In addition, harmine and harmol displaced [^{3}H]TCDD in the competitive ligand binding assay. At post-translational level, both harmine and harmol decreased the protein stability of CYP1A1, suggesting that posttranslational mechanism is involved. Furthermore, we demonstrated that the underlying mechanisms of the posttranslational modifications of both compounds involve ubiquitin-proteasomal pathway and direct inhibitory effects of CYP1A1 enzyme. We concluded that harmine and its metabolite, harmol, are new inhibitors of dioxin-mediated effects.

1. Introduction

Exposure to environmental contaminants such as polycyclic aromatic hydrocarbons (PAH) and halogenated aromatic hydrocarbons (HAH), including dioxins, plays an important role in the development of several types of human cancers. PAH and HAH are wide spread environmental contaminants that present in cigarette smoke, coal tar, automobile exhaust and charbroiled food. The carcinogenic effects of several PAH and HAH are initiated after their binding and activation of the aryl hydrocarbon receptor (AhR) (Mandal, 2005).

AhR is a ligand-activated transcription factor that is found inactive in the cytoplasm aggregated with two 90-KDa heat-shock proteins (HSP90), the co-chaperone p23 and a 43-KDa protein termed hepatitis B virus X-associated protein 2 (XAP2). Upon ligand binding, AhR gets activated and translocated to the nucleus where it heterodimerizes with another protein called AhR nuclear translocator (ARNT). This complex then binds to its DNA consensus sequence called xenobiotic responsive element (XRE) that is found in the upstream of the CYP1A1 and other AhR-responsive genes and hence stimulates transcription of these genes (Denison and Nagy, 2003).

CYP1A1 is a phase I xenobiotic metabolizing enzyme that bioactivates several PAH and other hydrophobic environmental procarcinogens into their ultimate carcinogenic forms (Shimada and Fujii-Kuriyama, 2004). CYP1A1 metabolizes PAH into reactive intermediates that form DNA adducts, which lead to mutagenesis and carcinogenesis (Shimada and Fujii-Kuriyama, 2004). Several lines of evidence demonstrate a strong correlation between the activity of CYP1A1 and the increased risk of different human cancers such as lung, colon and rectal cancers (Shah et al., 2009; Slattery et al., 2004). Therefore, the level of CYP1A1 is considered as a useful biomarker for the exposure to several carcinogens. Furthermore,

Abbreviations: Act-D, actinomycin D; AhR, aryl hydrocarbon receptor; βNF, β-naphthoflavone; CHX, cycloheximide; CYP, cytochrome P450; EMSA, electrophoretic mobility shift assay; 7ER, 7-ethoxyresorufin; EROD, 7-ethoxyresorufin O-deethylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HAH, halogenated aromatic hydrocarbons; 3MC, 3-methylcholanthrene; MG-132, carbobenzoxy-l-leucyl-l-leucyl-leucinal; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide); PAH, Polycyclic aromatic hydrocarbons; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; XRE, xenobiotic responsive element.

* Corresponding author at: Faculty of Pharmacy and Pharmaceutical Sciences, 3126 Dentistry/Pharmacy Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2N8. Tel.: +780 492 3071; fax: +780 492 1217.
E-mail address: aelkadi@pharmacy.ualberta.ca (A.O.S. El-Kadi).

0378-4274/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2011.09.030

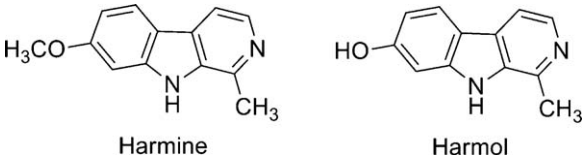

Fig. 1. Chemical structure of harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole), and harmol (1-methyl-9H-pyrido[3,4-b]indole-7-ol).

the inhibition of AhR activity and its regulated gene, CYP1A1 could result in the prevention of the toxic effects caused by the AhR ligands, including carcinogenicity (Puppala et al., 2008).

β-Carbolines are a large group of natural and synthetic indole alkaloids that are widely distributed in nature, including various foods, plants, marine creatures, insects, mammalians, as well as human tissues (Cao et al., 2007). β-Carbolines attracted considerable attention as they possess diverse pharmacological activities such as sedative, hypnotic, anxiolytic, anticonvulsant, antitumor, antithrombotic, antiparasitic, antimicrobial, as well as antiviral activities (Cao et al., 2007).

Harmine, 7-methoxy-1-methyl-9H-pyrido[3,4-b]indole and harmol, 1-methyl-9H-pyrido[3,4-b]indole-7-ol (Fig. 1), are β-carboline compounds that naturally found in several medicinal plants including *Peganum harmala* (Zygophyllaceae) and *Banisteriopsis Caapi* (Malpighiaceae) (Herraiz et al., 2010; Samoylenko et al., 2010). Harmine possesses several pharmacological activities such as antiplatelet aggregating, antimicrobial, antioxidant and antiprotozoal activities (Arshad et al., 2008; Di Giorgio et al., 2004; Im et al., 2009; Moura et al., 2007). Harmine can interact with several enzymes and neurotransmittors including topoisomerase I, 5-HT, monoamine oxidase-A, and cycline dependent kinases (Cao et al., 2005b, 2007; Herraiz et al., 2010; Song et al., 2004). Moreover, harmine is highly cytotoxic to several human tumor cell lines and showed promising antitumor effect for mice bearing tumor cells (Cao et al., 2005a). We previously demonstrated that *P. harmala* extract and its main active ingredient, harmine, inhibit the dioxin-mediated induction of Cyp1a1 at the catalytic activity level. Therefore, the aim of this study is to determine the effect of harmine and its main metabolite, harmol, on dioxin-mediated induction of CYP1A1 in human hepatoma HepG2 cells and to investigate the molecular mechanisms involved.

2. Material and methods

2.1. Chemicals and reagents

Cycloheximide (CHX), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 7-ethoxyresorufin (7ER), fluorescamine, harmine hydrochloride (>98% pure), 3-methylcholanthrene (3MC), β-naphthoflavone (βNF), and rabbit anti-goat IgG secondary antibody were purchased from Sigma–Aldrich (St. Louis, MO). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), >99% pure, was obtained from Cambridge Isotope Laboratories (Woburn, MA). TRIzol and Lipofectamine 2000 reagents were purchased from Invitrogen (Carlsbad, CA). Primary anti-mouse/human CYP1A1 antibody and primary goat anti-mouse/human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Santa Cruz (Santa Cruz, CA). Goat anti-mouse/human IgG secondary antibody was obtained from R&D systems (Minneapolis, MN). High-Capacity cDNA Reverse Transcription Kit and SYBR® Green PCR Master Mix were purchased from Applied Biosystems (Foster City, CA). Harmol hydrochloride (99% pure) was obtained from MP biomedicals (Solon, OH). Carbobenzoxy-l-leucyl-l-leucyl-leucinal (MG-132) and actinomycin D (Act-D) were purchased from Calbiochem (San Diego, CA). Chemiluminescence Western blotting detection reagents were obtained from GE Healthcare Life Sciences (Piscataway, NJ). [γ-^{32}P]-ATP was supplied by Perkin Elmer (Boston, MA). 2,3,7,8-Tetrachlorodibenzofuran (TCDF) and [^{3}H]-TCDD (13 Ci/mmol) were obtained from Dr. Stephen Safe (Texas A&M University). Dual-Luciferase Reporter Assay System was obtained from Promega Corporation (Madison, WI). All other chemicals were purchased from Fisher Scientific (Toronto, ON).

2.2. Animals and ethics

All experimental procedures involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. Male Hartley guinea pigs weighing 250–300 g and male C57BL/6 mice weighing 20–25 g were obtained from Charles River Canada (St. Constant, QC, Canada). All animals were exposed to 12 h light/dark cycles and were allowed free access to food and water.

2.3. Cell culture

Human hepatoma HepG2 and murine hepatoma Hepa 1c1c7 cell lines were purchased from American Type Culture Collection (Manassas, VA). Both cells were maintained in Dulbecco's modified Eagle's medium supplemented with heat-inactivated fetal bovine serum (10%, v/v), *L*-glutamine (2 mM), penicillin (100 IU/mL), and streptomycin (100 μg/mL). Cells were grown in 75-cm^2 tissue culture flasks at 37 °C in a 5% CO_2 humidified incubator.

2.4. Chemical treatments

Cells were treated in serum-free medium with TCDD in presence of various concentrations of harmine or harmol as specified under each experiment. Harmine, harmol, TCDD, MG-132, and Act-D were dissolved in dimethyl sulfoxide (DMSO) whereas CHX was dissolved in sterile distilled water. In all treatments, the DMSO concentration did not exceed 0.05% (v/v).

2.5. Determination of cell viability

The effect of harmine and harmol on HepG2 and Hepa 1c1c7 cell viability was determined by measuring the capacity of reducing enzymes present in viable cells to convert MTT to formazan crystals as described previously (Mosmann, 1983).

2.6. RNA isolation and real-time polymerase chain reaction (real-time PCR)

HepG2 cells and Hepa 1c1c7 were pre-incubated with increasing concentrations of harmine or harmol (0.5–12.5 μM) for 30 min before the addition of TCDD (1 nM) for 6 h. Thereafter, the total RNA was isolated using TRIzol reagent, according to the manufacturer's instructions (Invitrogen) as described previously (El Gendy and El-Kadi, 2009). Primers used in the current study were chosen from previous study (Anwar-Mohamed and El-Kadi, 2009); human CYP1A1: forward primer 5′-CGG CCC CGG CTC TCT-3′, reverse primer 5′-CGG AAG GTC TCC AGG ATG AA-3′, and human β-actin: forward primer 5′-CTG GCA CCC AGC ACA ATG-3′, reverse primer 5′-GCC GAT CCA CAC GGA GTA CT-3′, mouse Cyp1a1: forward primer 5′-GGT TAA CCA TGA CCG GGA ACT-3′, reverse primer 5′-TGC CCA AAC CAA AGA GAG TGA-3′, and mouse β-actin: forward primer 5′-TAT TGG CAA CGA GCG GTT CC-3′, reverse primer 5′-GGC ATA GAG GTC TTT ACG GAT GTC-3′. The primers were purchased from Integrated DNA technologies (IDT, Coralville, IA). Real-time PCR reactions were performed on an ABI 7500 instrument (Applied