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Posttranslational mechanisms modulating the expression of the cytochrome P450 1A1 gene by methylmercury in HepG2 cells: A role of heme oxygenase-1

Issa E.A. Amara, Anwar Anwar-Mohamed, Ayman O.S. El-Kadi*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E1

HIGHLIGHTS

- MeHg does not alter CYP1A1 mRNA or protein levels.
- MeHg significantly inhibited CYP1A1 activity.
- MeHg exerts its effect on CYP1A1 activity via HO-1.
- Inhibition or knockdown of HO-1 revert the effects of MeHg on CYP1A1 activity.
- MeHg induces NQO1 and HO-1 possibly through ARE.

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ABSTRACT

Recently we demonstrated the ability of mercuric chloride (Hg^{2+}) in human hepatoma HepG2 cells to significantly decrease the TCDD-mediated induction of Cytochrome P450 1A1 (CYP1A1) mRNA, protein, and catalytic activity levels. In this study we investigated the effect of methylmercury (MeHg) on CYP1A1 in HepG2 cells. For this purpose, cells were co-exposed to MeHg and TCDD and the expression of CYP1A1 mRNA, protein, and catalytic activity levels were determined. Our results showed that MeHg did not alter the TCDD-mediated induction of CYP1A1 mRNA, or protein levels; however it was able to significantly decrease CYP1A1 catalytic activity levels in a concentration-dependent manner. Importantly, this inhibition was specific to CYP1A1and was not radiated to other aryl hydrocarbon receptor (AhR)-regulated genes, as MeHg induced NAD(P)H:quinone oxidoreductase 1 mRNA and protein levels. Mechanistically, the inhibitory effect of MeHg on the induction of CYP1A1 coincided with an increase in heme oxygenase-1 (HO-1) mRNA levels. Furthermore, the inhibition of HO-1 activity, by tin mesoporphyrin, caused a complete restoration of MeHg-mediated inhibition of CYP1A1 activity, induced by TCDD. In addition, transfection of HepG2 cells with siRNA targeting the human HO-1 gene reversed the MeHg-mediated inhibition of TCDD-induced CYP1A1. In conclusion, this study demonstrated that MeHg inhibited the TCDD-mediated induction of CYP1A1 through a posttranslational mechanism and confirms the role of HO-1 in a MeHg-mediated effect.

1. Introduction

Cytochrome P450s (CYPs) are the major enzymes involved in xenobiotic metabolism accounting for around 75% of the total transformations of xenobiotics to either non-toxic or carcinogenic metabolites (Guengerich, 2008). Among these enzymes, CYP1A1 is of major interest because of its role in bioactivating procarcinogens and environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) into carcinogenic and mutagenic intermediates (Guengerich, 2004). Halogenated aromatic hydrocarbons (HAHs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are metabolically and chemically stable and highly lipophilic in the environment, with binding affinities in the pM to nM range (Denison and Nagy, 2003). Therefore, due to their persistence, HAHs are compounds of environmental concern. In this regard, it is well documented that CYP1A1 bioactivates PAHs to epoxide and diol-epoxide intermediates that subsequently lead to DNA and protein adducts formation (Shimada and Fujii-Kuriyama, 2004). HAHs can further enhance their carcinogenic effects by

Abbreviations: AhR, aryl hydrocarbon receptor; MeHg, methyl mercury chloride; CYPs, cytochrome P450s; HO-1, heme oxygenase-1; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); XRE, xenobiotic responsive element; ARE, antioxidant responsive element; SnMP, Tin mesoporphyrin; siRNA, short interference RNA.

* Corresponding author at: Faculty of Pharmacy and Pharmaceutical Sciences, 2142J Katz Group-Rexall Centre for Pharmacy and Health Research, University of Alberta, Edmonton, Alberta, Canada T6G 2E1. Tel.: +1 780 492 3071; fax: +1 780 492 1217.
E-mail address: aelkadi@ualberta.ca (A.O.S. El-Kadi).

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inducing the expression of the CYP1A1 gene, thereby increasing the levels of bioactivated PAHs intermediates (Nebert et al., 2004). The current knowledge of the mechanism of CYP1A1 induction by TCDD, the most potent CYP1A1 inducer tested to date (Mimura and Fujii-Kuriyama, 2003), suggests a transcriptional regulation, in which the binding of TCDD to a cytosolic transcription factor, the aryl hydrocarbon receptor (AhR), is the first step in a series of events leading to carcinogenicity and mutagenicity (Whitlock, 1999). The TCDD/AhR complex translocates to the nucleus where it heterodimerizes with the AhR nuclear translocator (ARNT) transcription factor, this whole complex of the TCDD/AhR/ARNT then binds to xenobiotic responsive elements (XRE) located in the promoter region of CYP1A1 resulting in the initiation of the mRNA transcription process (Pollenz, 2002; Whitlock, 1999).

One of the most common environmental problems that possesses multiple biological consequences, particularly to the xenobiotic metabolizing enzyme systems in the body is co-contamination with complex mixtures of HAHs and heavy metals (Amara et al., 2010). Heavy metals and HAHs are common contaminants of hazardous waste sites and are co-released from sources such as fossil fuel combustion, municipal waste incineration, and as components of tobacco smoke (McLemore et al., 1990). Both HAHs and heavy metals are ranked high on the list of the most hazardous xenobiotics in the environment, as reported by the Agency for Toxic Substances and Diseases Registry (ATSDR, 2011) and the Canadian Environmental Protection Act (CEPA, 2012).

Among the heavy metals, mercury is of potential interest since it is liberated from the earth's crust to the biosphere through degassing from volcanic areas or evaporation from the oceans in the form of elemental vapor (Hg°). The natural emissions are estimated to be between 2700 and 6000 tons per year (Lindberg et al., 1987). In addition, human activities have been estimated to add another 2000–3000 tons to the total release of mercury to the environment (Lindberg et al., 1987). Part of the emitted inorganic mercury becomes oxidized to Hg^{2+} and then methylated or transformed into organomercurials. The methylation is believed to involve a nonenzymatic reaction between Hg^{2+} and a methylcobalamine compound (analog of vitamin B_{12}) that is produced by bacteria (Wood and Wang, 1983). This reaction takes place primarily in aquatic systems. The intestinal bacterial flora of various animal species including fish is also, able to convert ionic mercury into methylmercuric (MeHg) compounds. MeHg is the most frequently encountered organic mercury compound in the environment (Wood and Wang, 1983). Moreover, increases in industrialization and changes in the environment during the twentieth century, has become one of the major sources that made humans and animals more exposed to numerous chemical forms of mercury, including MeHg (Fitzgerald and Clarkson, 1991). Inasmuch as mercury is ubiquitous in the environment, it is nearly impossible for most humans to avoid exposure to MeHg.

Previous reports, from our laboratory and others have demonstrated that inorganic mercury in the form of mercuric chloride (Hg^{2+}), alters the expression of the carcinogen-activating enzyme CYP1A1 at different stages along its signaling pathway and hence could affect the mutagenicity and carcinogenicity of HAHs (Amara et al., 2010; Korashy and El-Kadi, 2005). Therefore, the objective of the current study was to investigate whether the same effect on CYP1A1 expression gene would be observed upon exposure to MeHg in the human hepatoma HepG2 cells. To the best of our knowledge, this manuscript provides the first evidence for the ability of MeHg to reduce the TCDD-mediated induction of CYP1A1 by TCDD in HepG2 cells through a posttranslational mechanism.

2. Materials and methods

2.1. Material

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fluorescamine, anti-goat IgG peroxidase secondary antibody, protease inhibitor cocktail, and methyl mercury (II) chloride (MeHgCl) were purchased from Sigma Chemical Co. (St. Louis, MO). Tin mesoporphyrin (SnMP), was purchased from Frontier Scientific Inc. (Logan, UT). TCDD, >99% pure, was purchased from Cambridge Isotope Laboratories (Woburn, MA). TRIzol reagent and Lipofectamine 2000 reagents were purchased from Invitrogen (San Diego, CA). High-Capacity cDNA Reverse Transcription Kit, SYBR® Green PCR Master Mix, human Hmox1 (HO-1) validated siRNA was purchased from Applied Biosystems (Foster City, CA). INTERFERin siRNA transfecting reagent was purchased from Polyplus transfection (Illkirch, France). Actinomycin-D (Act-D) was purchased from Calbiochem (San Diego, CA). Chemiluminescence Western blotting detection reagents were from GE Healthcare Life Sciences (Piscataway, NJ). Nitrocellulose membrane was purchased from Bio-Rad Laboratories (Hercules, CA). CYP1A1 mouse polyclonal primary antibody, GAPDH rabbit polyclonal antibody, and anti-rabbit IgG peroxidase secondary antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-mouse IgG peroxidase secondary antibody was purchased from R&D Systems, Inc. (Minneapolis, MN). Luciferase assay reagents were obtained from Promega (Madison, WI). All other chemicals were purchased from Fisher Scientific (Toronto, ON).

2.2. Cell culture

HepG2 cell line, ATCC number HB-8065 (Manassas, VA), were maintained in Dulbecco's modified Eagle's medium (DMEM) with phenol red, supplemented with 10% heat-inactivated fetal bovine serum, 20 μM L-glutamine, 50 μg/ml amikacin, 100 IU/ml penicillin, 10 μg/ml streptomycin, 25 ng/ml amphotericin B, 0.1 mM nonessential amino acids, and vitamin supplement solution. Cells were grown in 75-cm^2 cell culture flasks at 37 °C in a 5% CO_2 humidified incubator.

2.3. Chemical treatments

Cells were treated in serum free medium with various concentrations of MeHg (1.25–5 μM) in the absence and presence of 1 nM TCDD, and/or 5 μM SnMP as described in figure legends. TCDD and SnMP were dissolved in dimethylsulfoxide (DMSO) and maintained in DMSO at −20 °C until use. MeHg (10 mM stock) was prepared freshly in double de-ionized water. In all treatments, the DMSO concentration did not exceed 0.05% (v/v).

2.4. Effect of MeHg on cell viability

The effect of MeHg on cell viability was determined using the MTT assay as described previously (Amara et al., 2010). MTT assay measures the conversion of MTT to formazan in viable cells. In brief, HepG2 cells were seeded onto 96-well microtiter cell culture plates and incubated for 24 h at 37 °C in a 5% CO_2 humidified incubator. Cells were treated with various concentrations of MeHg (1.25–20 μM) in the absence and presence of 1 nM TCDD. After 24 h incubation, the medium was removed and replaced with cell culture medium containing 1.2 mM MTT dissolved in phosphate buffered saline (PBS) (pH 7.4). After 2 h of incubation, the formed crystals were dissolved in isopropanol. The intensity of the color in each well was measured at a wavelength of 550 nm using the Bio-Tek EL 312e microplate reader (Bio-Tek Instruments, Winooski, VT).

2.5. RNA extraction and cDNA synthesis

Six hours after incubation with the test compounds, cells were collected and total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and quantified by measuring the absorbance at 260 nm. Thereafter, first-strand cDNA synthesis was performed using the High-Capacity cDNA reverse transcription kit (Applied Biosystems) according to the manufacturer's instructions. Briefly, 1.5 μg of total RNA from each sample was added to a mix of 2.0 μl 10× reverse transcription (RT) buffer, 0.8 μl 25× dNTP mix (100 mM), 2.0 μl 10× RT random primers, 1.0 μl MultiScribe reverse transcriptase, and 3.2 μl nuclease-free water. The final reaction mix was kept at 25 °C for 10 min, heated to 37 °C for 120 min, heated for 85 °C for 5 s, and finally cooled to 4 °C.

2.6. Quantification by real-time PCR

Quantitative analysis of specific mRNA expression was performed by real-time PCR, by subjecting the resulting cDNA to PCR amplification using 96-well optical reaction plates in the ABI Prism 7500 system (Applied Biosystems). Twenty-five-microliter reactions contained 0.1 μl of 10 μM forward primer and 0.1 μl of 10 μM reverse primer (40 nM final concentration of each primer), 12.5 μl of SYBR Green Universal Mastermix, 11.05 μl of nuclease-free water, and 1.25 μl of cDNA sample. The primers used in this study were chosen from previously published studies (Rushworth et al., 2008; Westerink and Schoonen, 2007) and are listed in Table 1.