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INORGANIC COMPOUNDS

Differential modulation of aryl hydrocarbon receptor regulated enzymes by arsenite in the kidney, lung, and heart of C57BL/6 mice

Anwar Anwar-Mohamed · Ghada Abdelhamid · Issa E. A. Amara · Ayman O. S. El-Kadi

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Abstract During the last couple of decades, efforts have been made to study the toxic effects of individual aryl hydrocarbon receptors (AhR) ligands such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or heavy metals typified by arsenic As(III). However, little is known about the combined toxic effects of TCDD and As(III) in vivo. Previous reports from our laboratory and others have demonstrated that As(III), by itself or in the presence of AhR ligands, such as TCDD, is capable of differentially altering the expression of various phase I and phase II AhR-regulated genes in in vitro systems. Thus, the objective of the current study was to investigate whether or not similar effects would occur at the in vivo level. Therefore, we examined the effect of exposure to As(III) (12.5 mg/kg) in the absence and presence of TCDD (15 μg/kg) on the AhR-regulated genes using C57Bl/6 mice. Our results demonstrated that As(III) alone inhibited Cyp1a1 and Cyp1a2 in the kidney, while it induced their levels in the lung and did not affect their mRNA levels in the heart. As(III) also induced Nqo1 and Gsta1 in all tested tissues. Upon co-exposure to As(III) and TCDD, As(III) inhibited the TCDD-mediated induction of Cyp1a1 in the kidney and heart, Cyp1a2 in the kidney and heart, while it potentiated TCDD-mediated induction of Cyp1a1 in the lung, and Nqo1 and Gsta1 in the kidney and lung. In conclusion, the present study demonstrates for the first time that As(III) modulates constitutive and TCDD-induced AhR-regulated genes in a time-, tissue-, and AhR-regulated enzyme-specific manner.

A. Anwar-Mohamed · G. Abdelhamid · I. E. A. Amara · A. O. S. El-Kadi (✉)
Faculty of Pharmacy and Pharmaceutical Sciences, 2142J Katz Group-Rexall Centre for Pharmacy and Health Research, University of Alberta, Edmonton, AB T6G 2N8, Canada
e-mail: aelkadi@pharmacy.ualberta.ca

Keywords AhR · Arsenite · Cyp1a1 · Cyp1a2 · Nqo1 · Gsta1

Abbreviations

AhR	Aryl hydrocarbon receptor
As(III)	Arsenite
Cyp450s	Cytochrome P450s
Gsta1	Glutathione-*S*-transferase A1
HO-1	Heme oxygenase-1
Nqo1	NADP(H): quinone oxidoreductase
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)
XRE	Xenobiotic responsive element

Introduction

The AhR is a member of basic-helix-loop-helix (bHLH)/Per-ARNT-Sim (PAS) family of transcription proteins. Inactive AhR resides in the cytoplasm bound to two 90-kDa heat shock proteins (HSP90), the 23-kDa heat shock protein (p23), and hepatitis B virus X-associated protein 2 (XAP2). Upon ligand binding, the AhR–ligand complex dissociates from the cytoplasmic complex and translocates to the nucleus where it associates with the aryl hydrocarbon receptor nuclear translocator (ARNT) (Nebert and Duffy 1997). The whole complex then acts as a transcription factor that binds to a specific DNA recognition sequence, termed the xenobiotic responsive element (XRE), located in the promoter region of a number of AhR-regulated genes. Among these genes are those encoding a number of xenobiotic metabolizing enzymes, including four phase I enzymes [cytochrome P450 1A1 (CYP1A1), CYP1A2, CYP1B1, and CYP2S1] and four phase II enzymes [NAD(P)H: quinone oxidoreductase-1 (NQO1),

glutathione-*S*-transferase A1 (GSTA1), cytosolic aldehyde dehydrogenase-3 (ADH3), and UDP-glucuronosyltransferase 1A6 (UGT1A6)] (Nebert and Duffy 1997; Rivera et al. 2002).

It has been previously postulated that the induction of phase II enzymes, such as Nqo1 and GSTA1, serves as a counterproductive detoxifying mechanism for many mutagens, carcinogens, and other toxic compounds that are generated by the phase I enzymes, typified by CYP1A1 and CYP1A2, in humans and rodents (Nebert and Dalton 2006). Several AhR ligands are not only agonists of the AhR, but, with the exception of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are also substrates for the induced phase I enzymes. The conversion of these AhR ligands into diol epoxide compounds by CYP1A1 and CYP1A2 results in the formation of covalent adducts when these genotoxic metabolites react with guanines in critical genes, potentially initiating tumorigenesis and other toxic responses (Spink et al. 2002). The coupling of phase II enzymes such as NQO1 and GSTA1 to these phase I enzymes is necessary to detoxify the generated toxic metabolites prior to their excretion (Nebert and Dalton 2006).

During the last couple of decades, efforts have been made to study the toxic effects of individual AhR ligands such as TCDD or heavy metals typified by arsenic. However, little is known about the combined toxic effects of TCDD and arsenic. In this context, arsenic and TCDD are ranked 1st and 5th, respectively, on the list of the most hazardous xenobiotics in the environment, as reported by the Agency for Toxic Substances and Diseases Registry and the Canadian Environmental Protection Act. (ATSDR 2011; CEPA 2012). Inorganic arsenic is released into the environment from primary copper, zinc, and lead smelters and glass, pesticide, and herbicide manufacturing (Elbekai and El-Kadi 2004). It is also found in cigarette smoke, arsenic-treated wood, and agricultural fertilizers (Patrick 2003). Exposure to arsenic might occur through the oral, inhalation, and dermal routes. Depending on the chemical form, 50–95 % of ingested arsenite (As(III)) or arsenate (As(V)) is absorbed through the oral route (Klaassen 2001). Following absorption, As(III) and As(V) are distributed evenly to all tissues (Elbekai and El-Kadi 2007, 2008). However, As(III) is far more toxic than As(V) (Cui et al. 2008), thus As(III) in the form of sodium arsenite has been utilized in the current study.

Previous reports from our laboratory and others have shown that As(III), by itself or in the presence of AhR ligands, is capable of differentially altering the expression of various phase I and phase II AhR-regulated genes in in vitro systems (Anwar-Mohamed et al. 2009). Most of these studies were conducted using the murine hepatoma Hepa 1c1c7 cells, human hepatoma HepG2 cells, and primary hepatocytes from rats and humans (Anwar-Mohamed et al. 2009). However, few studies examined the effect of As(III) in vivo using guinea pigs, rats, and mice models. Despite these efforts, these studies were inconclusive as they were either incomplete or just focused on the liver as a target organ to be studied (Anwar-Mohamed et al. 2009).

Although environmental co-exposures involve both AhR ligands, typified by TCDD, and heavy metals, typified by As(III), the current methods for assessing the potential toxicological consequences are often assuming the additivity of responses. However, this might not be the case, because there is still a possibility of antagonistic or synergistic effect. As such, emerging evidence suggests that TCDD and As(III) co-exposures generate different biological responses than is expected based on the toxicological mechanisms of each contaminant separately. Moreover, there has been no previous attempt to examine the effect of As(III) on the regulation of AhR-regulated genes in the presence and absence of TCDD in extrahepatic tissues. Therefore, we hypothesize that As(III) differentially alters the expression of AhR-regulated enzymes in a time-, tissue-, and AhR-regulated enzyme-specific manner. Thus, the objective of the current study was to investigate the time-, tissue-, and AhR-regulated enzyme-specific effects upon the exposure to a single dose of As(III) in the absence and presence of TCDD in extrahepatic tissues, namely, kidney, lung, and heart.

Materials and methods

Materials

TRIzol reagent was purchased from Invitrogen (Carlsbad, CA). High-Capacity cDNA Reverse Transcription Kit, SYBR Green SuperMix and 96-well optical reaction plates with optical adhesive films were purchased from Applied Biosystems (Foster City, CA). Real-time PCR primers were synthesized by Integrated DNA Technologies Inc. 7-ethoxyresorufin, anti-goat IgG peroxidase secondary antibody, protease inhibitor cocktail, and sodium arsenite ($NaAsO_2$) were purchased from Sigma Chemical Co. (St. Louis, MO). TCDD, >99 % pure, was purchased from Cambridge Isotope Laboratories (Woburn, MA). Chemiluminescence Western blotting detection reagents were from GE Healthcare Life Sciences (Piscataway, NJ). Nitrocellulose membrane was purchased from Bio-Rad Laboratories (Hercules, CA). Cyp1a1/1a2 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). Cyp1b1 polyclonal primary antibody was purchased from BD Biosciences (Mississauga, ON). Gsta1 primary polyclonal antibody was purchased from Abcam (Cambridge, MA). Nqo1 polyclonal primary antibody and anti-mouse IgG

peroxidase secondary antibody were purchased from R&D Systems, Inc. (Minneapolis, MN). All other chemicals were purchased from Fisher Scientific (Toronto, ON).

Mice handling

Male C57BL6 mice, aged 10–12 weeks (Charles River Laboratories), were used in this study. Mice were group-housed under standard conditions, three to five per cage with food and water available ad libitum, and were maintained on a 12-h light/dark cycle. Mice were treated in compliance with University of Alberta Health Sciences Animal Policy and Welfare Committee guidelines. All experiments included matched numbers of male mice.

Biohazard precaution

TCDD is a toxic and a likely human carcinogen. All personnel were instructed as to safe handling procedures. Laboratory coats, gloves, and masks were worn at all times, and contaminated materials were collected separately for disposal by the Office of Environmental Health and Safety at the University of Alberta.

Animal treatment

Male C57BL/6 (22–30 g) mice were obtained from Charles River, Canada (Montreal, QC, Canada). Animals were injected intraperitoneally (i.p.) with As(III) (as sodium arsenite dissolved in saline) at 12.5 mg/kg in the absence and presence of 15 μg/kg TCDD (dissolved in corn oil) injected i.p. The mice were divided into 4 groups. The first group ($n = 12$) control mice received saline (0.4 mL) plus corn oil (0.4 mL). The second group ($n = 12$) As(III)-treated mice received As(III) dissolved in saline (0.4 mL) plus corn oil (0.4 mL). The third group ($n = 12$) TCDD-treated mice received TCDD dissolved in corn oil (0.4 mL) plus saline (0.4 mL). The fourth group ($n = 12$) As(III)-plus TCDD-treated mice received As(III) dissolved in saline (0.4 mL) plus TCDD dissolved in corn oil (0.4 mL). Thereafter, the animals were euthanized after a single injection at 6 h ($n = 6$) and 24 h ($n = 6$) via cervical dislocation. Heart, lung, and kidney tissues were excised, immediately frozen in liquid nitrogen, and stored at −80 °C until further analysis. All experimental procedures involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee.

RNA extraction and cDNA synthesis

Total RNA from the frozen tissues was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and quantified by measuring the absorbance at 260 nm. RNA quality was determined by measuring the 260/280 ratio. 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 of 10× reverse transcriptase buffer, 0.8 μL of 25× dNTP mix (100 mM), 2.0 μL of 10× reverse transcriptase random primers, 1.0 μL of MultiScribe reverse transcriptase, and 3.2 μL of nuclease-free water. The final reaction mix was kept at 25 °C for 10 min, heated to 37 °C for 120 min, heated to 85 °C for 5 s, and finally cooled to 4 °C.

Quantification by real-time PCR

Quantitative analysis of specific mRNA expression was performed using 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). The 25-μL reaction mix 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), list of primers is shown in Table 1, 12.5 μL of SYBR Green Universal Mastermix, 11.05 μL of nuclease-free water, and 1.25 μL of cDNA sample. Assay controls were incorporated onto the same plate, namely, no-template controls to test for the contamination of any assay reagents. After sealing the plate with an optical adhesive cover, the thermocycling conditions were initiated at 95 °C for 10 min, followed by 40 PCR cycles of denaturation at 95 °C for 15 s, and

Table 1 Primers sequences used for real-time PCRs

Genes	Forward primers	Reverse primers
B-actin	5′-TAT TGG CAA CGA GCG GTT CC-3′	5′-GGC ATA GAG GTC TTT ACG GAT GTC-3′
Cyp1a1	5′-GGT TAA CCA TGA CCG GGA ACT-3′	5′-TGC CCA AAC CAA AGA GAG TGA-3′
Cyp1a2	5′-TGG AGC TGG CTT TGA CAC AG-3′	5′-CGT TAG GCC ATG TCA CAA GTA GC-3′
Gsta1	5′-CCC CTT TCC CTC TGC TGA AG-3′	5′-TGC AGC TTC ACT GAA TCT TGA AAG-3′
Nqo1	5′-GGA AGC TGC AGA CCT GGT GA-3′	5′-CCT TTC AGA ATG GCT GGC A-3′
HO-1	5′-GTG ATG GAG CGT CCA CAG C-3′	5′-TGG TGG CCT CCT TCA AGG-3′

annealing/extension at 60 °C for 1 min. Melting curve (dissociation stage) was performed by the end of each cycle to ascertain the specificity of the primers and the purity of the final PCR product.

Real-time PCR data analysis

The real-time PCR data were analyzed using the relative gene expression, that is, ($\Delta\Delta C_T$) method as described in Applied Biosystems User Bulletin No.2 and explained further by Livak and Schmittgen (Livak and Schmittgen 2001). Briefly, the ΔC_T values were calculated in every sample for each gene of interest as follows: $C_{T\ \text{gene of interest}} - C_{T\ \text{reporter gene}}$, with β-actin as the reporter gene. Calculation of relative changes in the expression level of one specific gene ($\Delta\Delta C_T$) was performed by subtraction of ΔC_T of control (vehicle-treated animals at 6- or 24-h time points) from the ΔC_T of the corresponding treatment groups. The values and ranges given in different figures were determined as follows: $2^{-\Delta(\Delta CT)}$ with $\Delta\Delta C_T + SE$ and $\Delta\Delta C_T - SE$, where SE is the standard error of the mean of the $\Delta(\Delta C_T)$ value.

Preparation of microsomal and cytosolic protein fractions

Lung and kidney microsomes were prepared by differential centrifugation of homogenized tissues as previously described (Lin et al. 1991; Barakat et al. 2001). Briefly, individual lung and kidney tissues were rapidly removed and washed in ice-cold potassium chloride [1.15 % (w/v)]. Consequently, they cut into pieces and homogenized separately in cold sucrose solution (1 g of tissue in 5 mL of 0.25 M sucrose). After homogenizing, the tissues were separated by different ultracentrifugation. The final pellets were reconstituted in cold sucrose, and the supernatant cytosols, were all stored at −80 °C. Thereafter, microsomal and cytosolic protein concentrations were determined by the Lowry method using bovine serum albumin as a standard (Lowry et al. 1951).

Western blot analysis

Western blot analysis was performed using a previously described method (Zordoky et al. 2010). Briefly, 20 μg of kidney and lung microsomal or cytosolic proteins was separated by 10 % sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE), and then electrophoretically transferred to nitrocellulose membrane. Protein blots were then blocked overnight at 4 °C in blocking solution containing 0.15 M sodium chloride, 3 mM potassium chloride, 25 mM Tris–base (TBS), 5 % skim milk, 2 % bovine serum albumin, and 0.5 % Tween 20. After blocking, the blots were incubated with the following primaries: primary polyclonal mouse anti-rat Cyp1a1/1a2, primary polyclonal goat anti-mouse Nqo1, primary polyclonal goat anti-rat Gsta1, primary polyclonal rabbit anti-mouse actin, or primary polyclonal goat anti-mouse Gapdh for 2 h at room temperature. Incubation with a peroxidase-conjugated goat anti-rabbit IgG secondary antibody for Nqo1 and actin or goat anti-mouse IgG secondary antibody for Cyp1a1/1a2, or rabbit anti-goat IgG secondary antibody for Gsta1 and Gapdh was carried out for another 2 h at room temperature. The bands were visualized using the enhanced chemiluminescence method according to the manufacturer's instructions (GE Healthcare Life Sciences, Piscataway, NJ). The intensity of the protein bands was quantified, relative to the signals obtained for actin, using ImageJ software (National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij).

Microsomal incubation and measuring EROD and MROD catalytic activities

Microsomes from lung and kidney of different treatments (1 mg protein/mL) were incubated in the incubation buffer (5 mM magnesium chloride hexahydrate dissolved in 0.5 M potassium phosphate buffer pH = 7.4) at 37 °C in a shaking water bath (50 rpm). A pre-equilibration period of 5 min was performed. The reaction was initiated by the addition of 1 mM NADPH. The concentrations of substrate were 2 μM for 7-ethoxyresorufin (EROD) and 7-methoxyresorufin (MROD). After incubation at 37 °C (5 min for EROD and 10 min for MROD assays), the reaction was stopped by adding 0.5 mL of cold methanol. The amount of resorufin formed in the resulting supernatant was measured using the Baxter 96-well fluorescence plate reader using excitation and emission wavelengths of 545 and 575 nm, respectively. Formation of resorufin was linear with incubation time and protein amount. Enzymatic activities were expressed as picomole of resorufin formed per minute and per milligram of microsomal proteins.

Determination of Nqo1 enzymatic activity

The Nqo1 activity was determined by the continuous spectrophotometric assay to quantitate the reduction in its substrate, 2,6-dichlorophenolindophenol (DCPIP) as described previously (Korashy and El-Kadi 2006; Preusch et al. 1991). Briefly, 20 μg of lung and kidney cytosolic protein of different treatments was incubated with 1 mL of the assay buffer (40 μM DCPIP, 0.2 mM NADPH, 25 mM Tris–HCl, pH 7.8, 0.1 % (v/v) Tween 20, and 0.7 mg/mL bovine serum albumin, 0 or 30 μM dicoumarol). The rate of DCPIP reduction was monitored over 90 s at 600 nm with an extinction coefficient (ε) of 2.1 $mM^{-1}\ cm^{-1}$. The Nqo1 activity was calculated as the decrease in absorbance

per min per mg of total protein of the sample that quantitates the dicoumarol-inhibitable reduction in DCPIP.

Determination of GST activity

GST activity was determined spectrophotometrically using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to the method of Habig (Habig et al. 1974). Briefly, 20 μg of lung and kidney cytosolic protein of different treatments was incubated with 1 mM CDNB, 1 mM reduced glutathione in 0.1 M potassium phosphate buffer, pH 6.5 at 25 °C in a total volume of 1 mL. GST activity was measured as the amount of CDNB conjugate formed by recording the absorbance at 340 nm for 1.5 min with an extinction coefficient of 9,600 M^{-1}/cm^{-1}. The enzyme activity was expressed as nmol/min/mg protein.

Statistical analysis

Data are presented as mean ± standard error of the mean. Control and treatment measurements were compared using Student's *t* test. Comparative gene expression across tissues was analyzed using a one-way analysis of variance followed by a Student–Newman–Keuls post hoc comparison. The result was considered statistically significant when $P < 0.05$.

Results

Differential expression of AhR-regulated genes and HO-1 in the kidney, lung, and heart

All of the genes examined were found to be constitutively expressed in the kidney, lung, and heart at different levels (Figs. 1, 2). Cyp1a1 expression differed greatly across the extrahepatic organs with the lung being the highest expressing tissue followed by the kidney and lastly the heart (Fig. 1a). Similarly, Cyp1a2 expression showed a similar trend to that of Cyp1a1 with the lung being the highest expressing tissue followed by the kidney and lastly the heart (Fig. 1b). The phase II AhR-regulated genes showed a different expression pattern across tissues than phase I AhR-regulated genes. For example, Nqo1 gene expression was the highest in the kidney followed by the heart and lastly the lung (Fig. 2a). On the contrary, Gsta1 expression was the highest in the lung followed by the kidney and lastly the heart (Fig. 2b). Aside from the phase II AhR-regulated enzymes, HO-1, which is mainly regulated by the nuclear factor erythroid 2 p45-related factor 2 (Nrf2)/antioxidant responsive element (ARE) signaling pathway, expression was the highest in the kidney followed by the lung and the heart (Fig. 2c).

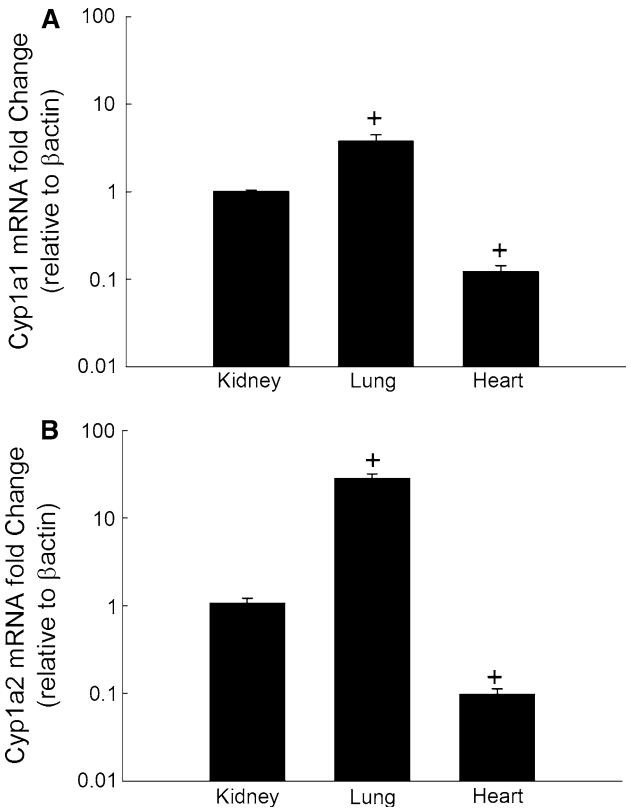


Fig. 1 Constitutive expression of phase I AhR-regulated genes in different tissues. Total RNA was isolated from different kidney, lung, and heart of 6-h time point control animals. The relative expression of Cyp1a1 (**a**) and Cyp1a2 (**b**) was determined by reverse transcription followed by real-time PCR. The data were analyzed using the relative gene expression method. The data were normalized to the endogenous reference gene (*β*-actin) and relative to a calibrator (kidney). Results are presented as mean ± SEM ($n = 6$), $+P < 0.05$, compared to kidney

Effect of co-exposure to As(III) and TCDD on Cyp1a1 and Cyp1a2 mRNA in the kidney, lung, and heart of C57Bl6 mice

At 6 h and 24 h, As(III) alone significantly induced lung Cyp1a1 mRNA levels by 10- and 0.80-fold, respectively, compared to control (Fig. 3a, b). In kidney and heart, As(III) alone failed to cause any significant changes in Cyp1a1 mRNA levels at 6 h and 24 h (Fig. 3a, b). TCDD alone significantly induced Cyp1a1 mRNA levels at 6 h in the kidney, lung, and heart by 880-, 180-, and 420-fold, respectively, and at 24 h by 270-, 80-, and 415-, respectively, compared to control (Fig. 3a, b). When animals were co-exposed to As(III) and TCDD, As(III) at 6 h significantly inhibited the TCDD-mediated induction of Cyp1a1 mRNA in the kidney and heart by 612- and 148-fold, respectively, compared to TCDD alone. On the other hand, As(III) at 6 h significantly potentiated the TCDD-mediated induction of Cyp1a1 mRNA in the lung by 109-fold compared to TCDD alone (Fig. 3a). As(III) at

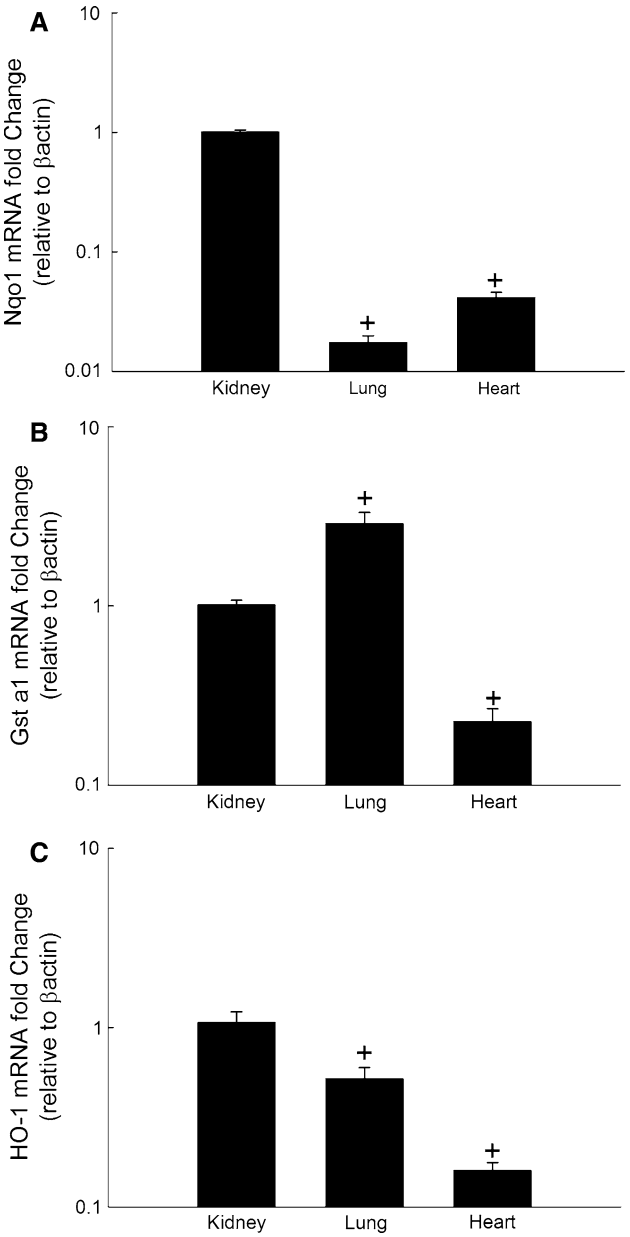


Fig. 2 Constitutive expression of phase II AhR-regulated genes and HO-1 in different tissues. Total RNA was isolated from different kidney, lung, and heart of 6-h time point control animals. The relative expression of Nqo1 (**a**), Gsta1 (**b**), and HO-1 (**c**) was determined by reverse transcription followed by real-time PCR. The data were analyzed using the relative gene expression method. The data were normalized to the endogenous reference gene (β-actin) and relative to a calibrator (kidney). Results are presented as mean ± SEM ($n = 6$), $+P < 0.05$, compared to kidney

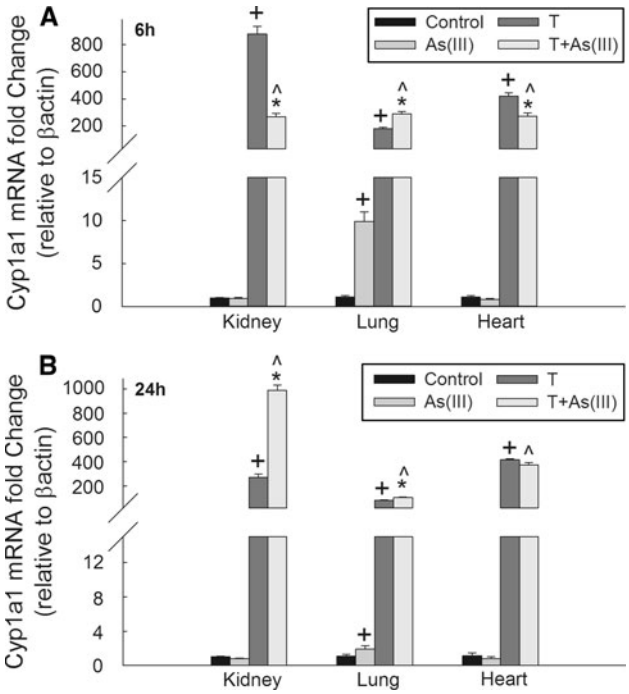


Fig. 3 Effect of co-exposure to As(III) and TCDD on Cyp1a1 mRNA in the kidney, lung, and heart of C57Bl6 mice. Animals were injected i.p. with 12.5 mg/kg As(III) in the absence and presence of 15 μg/kg TCDD for 6 h (**a**) and 24 h (**b**). First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from kidney, and the expression of Cyp1a1 was measured using real-time PCR. cDNA fragments were amplified and quantitated using ABI 7500 real-time PCR system as described under "Materials and methods". Duplicate reactions were performed for each experiment, and the values presented are the means of six independent experiments. $^{+}P < 0.05$, compared to control (untreated animals); $^{*}P < 0.05$, compared to respective TCDD treatment; $^{\wedge}P < 0.05$, compared to respective As(III) treatment

24 h significantly potentiated kidney and lung Cyp1a1 mRNA levels by 719- and 23-fold, respectively, compared to TCDD alone, while it did not alter the TCDD-mediated induction of Cyp1a1 mRNA in the heart (Fig. 3b).

At 6 h and 24 h, As(III) alone failed to significantly affect Cyp1a2 mRNA levels in kidney, lung, or heart (Fig. 4a, b). TCDD alone significantly induced Cyp1a2 mRNA levels at 6 h in the kidney, lung, and heart by 40-, 0.80-, and 12-fold, respectively, and at 24 h by 13-, 0.80-, and 12-fold, respectively, compared to control (Fig. 4a, b). When animals were co-exposed to As(III) and TCDD, As(III) at 6 h significantly inhibited the TCDD-mediated induction of Cyp1a2 mRNA in the kidney and heart by 25- and 8.50-fold, respectively, compared to TCDD alone. On the other hand, As(III) at 6 h did not alter the TCDD-mediated induction of Cyp1a2 mRNA in the lung (Fig. 4a). In contrast, As(III) at 24 h significantly potentiated kidney Cyp1a2 mRNA by sevenfold, compared to TCDD alone, while it did not alter the TCDD-mediated induction of Cyp1a2 mRNA in the lung and heart (Fig. 4b).

Effect of co-exposure to As(III) and TCDD on Cyp1a protein expression and EROD and MROD catalytic activity levels in the kidney and lung of C57Bl/6 mice

Our results showed that As(III) alone significantly induced lung Cyp1a protein expression levels by 2.30-fold

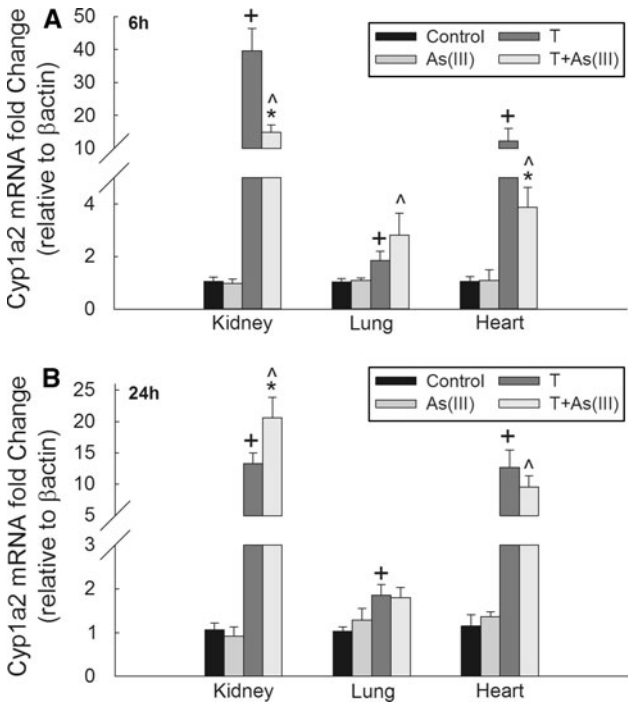


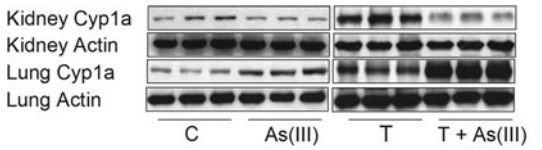
Fig. 4 Effect of co-exposure to As(III) and TCDD on Cyp1a2 mRNA in the kidney, lung, and heart of C57Bl6 mice. Animals were injected i.p. with 12.5 mg/kg As(III) in the absence and presence of 15 μg/kg TCDD for 6 h (**a**) and 24 h (**b**). First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from kidney, and the expression of Cyp1a2 was measured using real-time PCR. cDNA fragments were amplified and quantitated using ABI 7500 real-time PCR system as described under "Materials and methods". Duplicate reactions were performed for each experiment, and the values presented are the means of six independent experiments. $^{+}P < 0.05$, compared to control (untreated animals); $*P < 0.05$, compared to respective TCDD treatment; $\wedge P < 0.05$, compared to respective As(III) treatment

compared to control (Fig. 5). TCDD alone significantly induced kidney and the lung Cyp1a protein expression levels by 7.50- and 11-fold, respectively, compared to control (Fig. 5). On the other hand, when animals were co-exposed to As(III) and TCDD, As(III) significantly inhibited TCDD-mediated induction of kidney Cyp1a protein expression levels by sevenfold, while on the other hand, it significantly potentiated the TCDD-mediated induction of lung Cyp1a protein expression levels by 138-fold compared to TCDD alone (Fig. 5).

At the catalytic activity levels, As(III) alone significantly inhibited the kidney EROD activity by 0.50-fold, while it significantly induced the lung EROD activity by sevenfold (Fig. 6a). Similarly, As(III) alone significantly inhibited the kidney MROD activity by 0.70-fold, while it significantly induced the lung MROD activity by 2.20-fold, respectively, compared to control (Fig. 6b). TCDD alone significantly induced kidney and lung EROD activities by 11.50- and 13-fold, and kidney and lung MROD activities by 14- and 0.80-fold, respectively, compared to control (Fig. 6a, b). When animals were co-exposed to As(III) and TCDD, As(III) significantly inhibited TCDD-mediated induction of kidney EROD catalytic activity by 5.50-fold, while on the other hand, it potentiated the TCDD-mediated induction of lung EROD by 127-fold, respectively, compared to TCDD alone (Fig. 6a). Moreover, As(III) significantly inhibited the TCDD-mediated induction of kidney MROD catalytic activity by 21-fold, while on the other hand, it potentiated the TCDD-mediated induction of lung MROD catalytic activity by 21-fold, respectively, compared to TCDD alone (Fig. 6b).

Effect of co-exposure to As(III) and TCDD on Nqo1 and Gsta1 mRNA in the kidney, lung, and heart of C57Bl6 mice

At 6 h, As(III) alone significantly induced the kidney, lung, and heart Nqo1 mRNA levels by threefold, fourfold, and twofold, respectively, compared to control (Fig. 7a). At 24 h, As(III) alone significantly induced the kidney, lung,



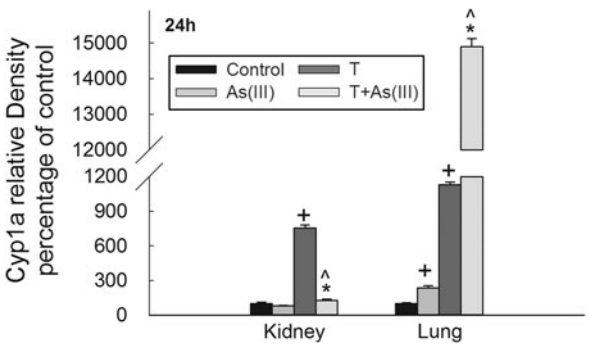


Fig. 5 Effect of co-exposure to As(III) and TCDD on Cyp1a protein expression levels in the kidney and lung of C57Bl/6 mice. Kidney and lung microsomal proteins were isolated after 24 h of treatment. 30 μg of microsomal proteins was separated on a 10 % SDS-PAGE. Proteins were detected using the enhanced chemiluminescence method. The *graph* represents the relative amount of protein normalized to *β*-actin signals (mean ± SEM, $n = 6$), and the results are expressed as percentage of the control values taken as 100 %. $^{+}P < 0.05$, compared to control; $*P < 0.05$, compared to respective TCDD treatment; $\wedge P < 0.05$, compared to respective As(III) treatment

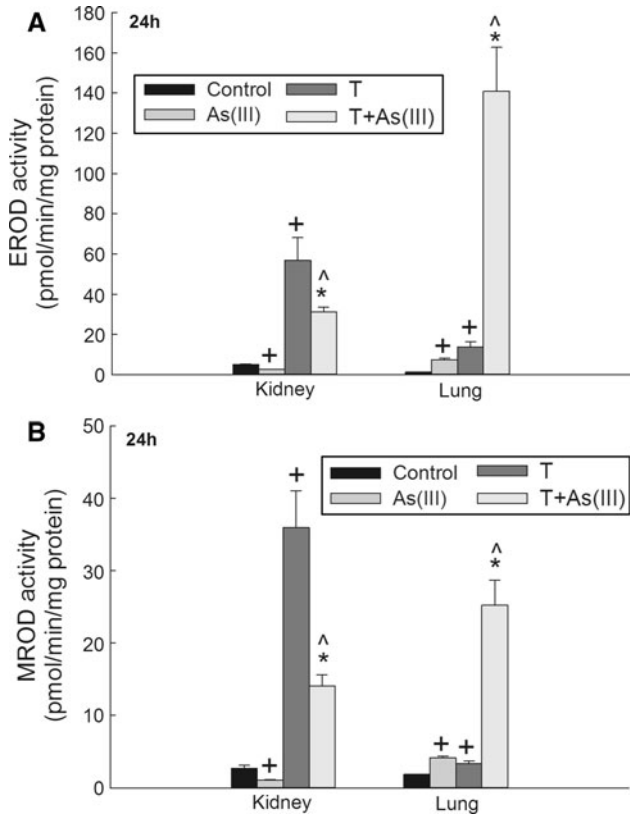


Fig. 6 Effect of co-exposure to As(III) and TCDD on EROD and MROD catalytic activity levels in the kidney and lung of C57Bl/6 mice. Kidney and lung microsomal proteins were isolated after 24 h of treatment. EROD and MROD activities were measured using 7-ethoxyresorufin and 7-methoxyresorufin as substrates, respectively. The reaction was started by the addition of 1 mM NADPH and lasted for 5 min for EROD and 10 min for MROD. The reaction was terminated by the addition of ice-cold acetonitrile. Values are presented as mean ± SEM ($n = 6$). $^{+}P < 0.05$, compared to control; $^{*}P < 0.05$, compared to respective TCDD treatment; $^{\wedge}P < 0.05$, compared to respective As(III) treatment

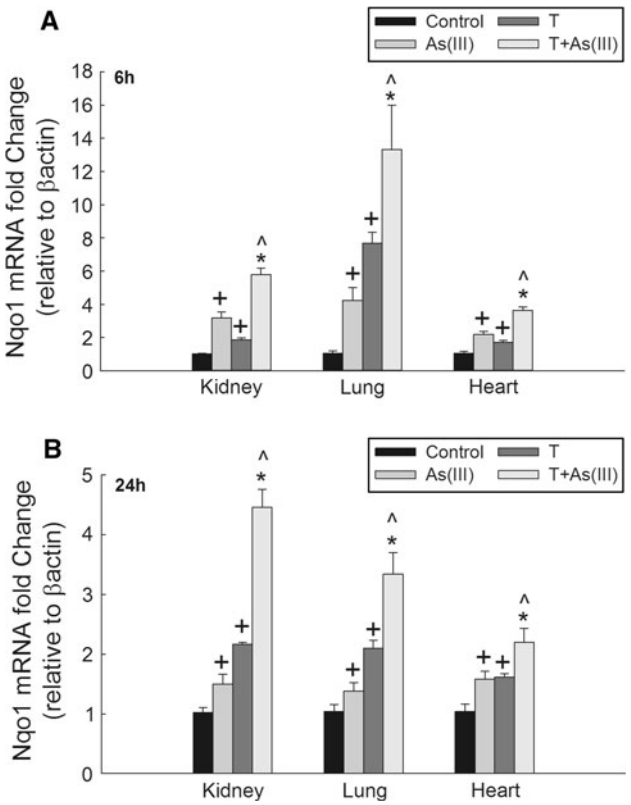


Fig. 7 Effect of co-exposure to As(III) and TCDD on Nqo1 mRNA in the kidney, lung, and heart of C57Bl6 mice. Animals were injected i.p. with 12.5 mg/kg As(III) in the absence and presence of 15 μg/kg TCDD for 6 h (**a**) and 24 h (**b**). First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from kidney, and the expression of Nqo1 was measured using real-time PCR. cDNA fragments were amplified and quantitated using ABI 7500 real-time PCR system as described under "Materials and methods". Duplicate reactions were performed for each experiment, and the values presented are the means of six independent experiments. $^{+}P < 0.05$, compared to control (untreated animals); $^{*}P < 0.05$, compared to respective TCDD treatment; $^{\wedge}P < 0.05$, compared to respective As(III) treatment

and heart Nqo1 mRNA levels by 1.50-, 1.40-, and 1.60-fold, respectively, compared to control (Fig. 7b). TCDD alone significantly induced Nqo1 mRNA levels at 6 h in the kidney, lung, and heart by 1.80-, 7.70-, and 1.70-fold, respectively, and at 24 h by 2-, 2-, and 1.60-fold, respectively, compared to control (Fig. 7a, b). When animals were co-exposed to As(III) and TCDD, As(III) at 6 h significantly potentiated the TCDD-mediated induction of Nqo1 mRNA in the kidney, lung, and heart by 4-, 5.70-, and 2-fold, respectively, compared to TCDD alone (Fig. 7a). Similarly, As(III) at 24 h significantly potentiated the kidney, lung, and heart Nqo1 mRNA levels by 2.29-, 1.30-, and 0.50-fold, respectively, compared to TCDD alone (Fig. 7b).

At 6 h, As(III) alone significantly induced the kidney, lung, and heart Gsta1 mRNA levels by 598-, 31-, and 49-fold, respectively, compared to control (Fig. 8a). At 24 h, As(III) alone significantly induced kidney, lung, and heart Gsta1 mRNA levels by 206-, 7-, and 10-fold, respectively, compared to control (Fig. 8b). TCDD alone significantly induced Gsta1 mRNA levels at 6 h in the kidney, lung, and heart by 8.90-, 2.20-, and 2.50-fold, respectively, compared to control (Fig. 8a). At 24 h, TCDD alone significantly induced Gsta1 mRNA levels in kidney and heart by 6.20- and 2.90-fold, respectively, compared to control, while it did not alter lung Gsta1 mRNA levels (Fig. 8b). When animals were co-exposed to As(III) and TCDD, As(III) at 6 h significantly potentiated the TCDD-mediated induction of Gsta1 mRNA in the kidney, lung, and heart by 890-, 27-, and 91-fold, respectively, compared to TCDD alone (Fig. 8a). Similarly, As(III) at 24 h significantly potentiated the kidney, lung, and heart Gsta1 mRNA levels by 344-, 4.60-, and 21-fold, respectively, compared to TCDD alone (Fig. 8b).

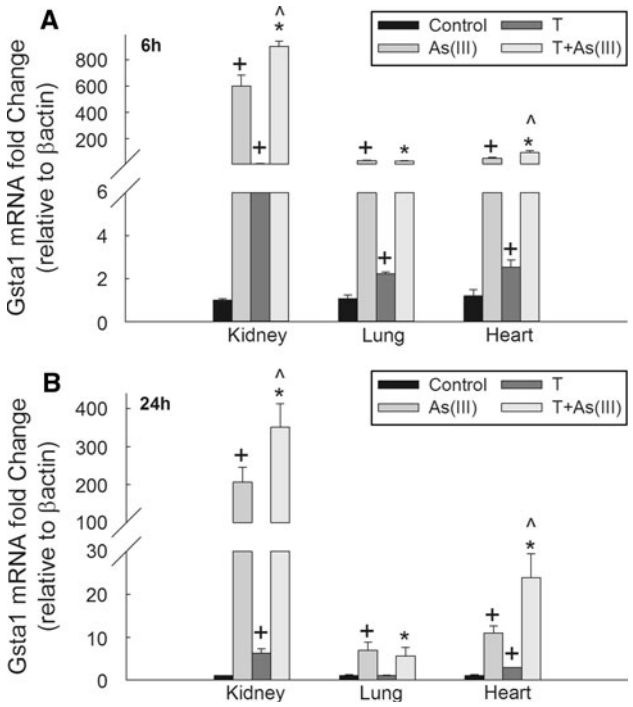


Fig. 8 Effect of co-exposure to As(III) and TCDD on Gsta1 mRNA in the kidney, lung, and heart of C57Bl6 mice. Animals were injected i.p. with 12.5 mg/kg As(III) in the absence and presence of 15 μg/kg TCDD for 6 h (**a**) and 24 h (**b**). First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from kidney, and the expression of Gsta1 was measured using real-time PCR. cDNA fragments were amplified and quantitated using ABI 7500 real-time PCR system as described under "Materials and methods". Duplicate reactions were performed for each experiment, and the values presented are the means of six independent experiments. $^{+}P < 0.05$, compared to control (untreated animals); $^{*}P < 0.05$, compared to respective TCDD treatment; $^{\wedge}P < 0.05$, compared to respective As(III) treatment

Effect of co-exposure to As(III) and TCDD on Nqo1 and Gsta1 protein expression and catalytic activity levels in the kidney and lung of C57Bl6 mice

As(III) alone significantly induced the kidney and lung Nqo1 protein expression levels by 6.50- and 6-fold, respectively, and kidney and lung Gsta1 protein expression levels by 10- and 5-fold, respectively, compared to control (Fig. 9a, b). TCDD alone significantly induced kidney and lung Nqo1 protein expression levels by 0.60- and 5-fold, respectively, and kidney and lung Gsta1 protein expression levels by 3.40- and 2.30-fold, respectively, compared to control (Fig. 9a, b). When animals were co-exposed to As(III) and TCDD, As(III) significantly potentiated the TCDD-mediated induction of kidney and lung Nqo1 protein expression levels by 6.80- and 7.50-fold, respectively, and kidney and lung Gsta1 protein expression levels by 13- and 0.32-fold, respectively, compared to TCDD alone (Fig. 9a, b).

At the catalytic activity levels, As(III) alone significantly induced the kidney and lung Nqo1 activities by 1.95- and 1.95-fold, respectively, compared to control (Fig. 10a). Similarly, As(III) alone significantly induced kidney and lung Gsta1 activities by 1.60- and 2-fold, respectively, compared to control (Fig. 10a). TCDD alone significantly induced lung Nqo1 activity by 1.50-fold, compared to control, while it did not alter the kidney Nqo1 activity (Fig. 10a). Furthermore, TCDD alone significantly induced kidney and lung Gsta1 activities by 1.20- and 1.80-fold, respectively, compared to control (Fig. 10b). When animals were co-exposed to As(III) and TCDD, As(III) significantly potentiated the TCDD-mediated induction of the kidney and lung Nqo1 catalytic activities by 2- and 0.50-fold, respectively, compared to TCDD alone. Additionally, As(III) significantly potentiated the TCDD-mediated induction of kidney Gsta1 catalytic activity by 0.32-fold, compared to TCDD alone, while it did not alter the TCDD-mediated induction of lung Gsta1 catalytic activity (Fig. 10b).

Effect of co-exposure to As(III) and TCDD on HO-1 mRNA levels in the kidney, lung, and heart

Our results demonstrated that As(III) alone was able to induce HO-1 mRNA levels in the kidney, lung, and heart after 6 h of treatment by 120-, 53-, 234-fold, respectively, compared to control (Fig. 11a). Importantly, As(III) alone after 24 h of treatment significantly induced lung HO-1 mRNA levels by 2.20-fold, while it did not significantly affect HO-1 mRNA levels in the kidney or heart (Fig. 11b). TCDD alone, after 6 h of treatment, significantly induced HO-1 mRNA levels only in the heart by 2-fold, compared to control (Fig. 11a). When the animals were co-exposed to As(III) and TCDD for 6 h, HO-1 mRNA levels were not significantly different from those of As(III) alone (Fig. 11a), yet this induction was not significantly different from that of As(III) alone. At 24 h, however, HO-1 mRNA levels were significantly induced in the kidney, lung, and heart by 1.70-, 2-, and 2.50-fold, respectively, compared to control (Fig. 11b).

Discussion

In the current study, we have demonstrated that As(III) modulates the constitutive and TCDD-inducible AhR-regulated enzymes in a time-, tissue-, and AhR-regulated enzyme-specific manner (Table 2). Furthermore, the concentrations of As(III) and TCDD utilized in the current study were selected based on previous in vivo studies that used the same concentrations of both As(III) and TCDD on C57BL/6 mice (Mehra and Kanwar 1980; Hu et al. 1999; Uno et al. 2008; Wong et al. 2010).

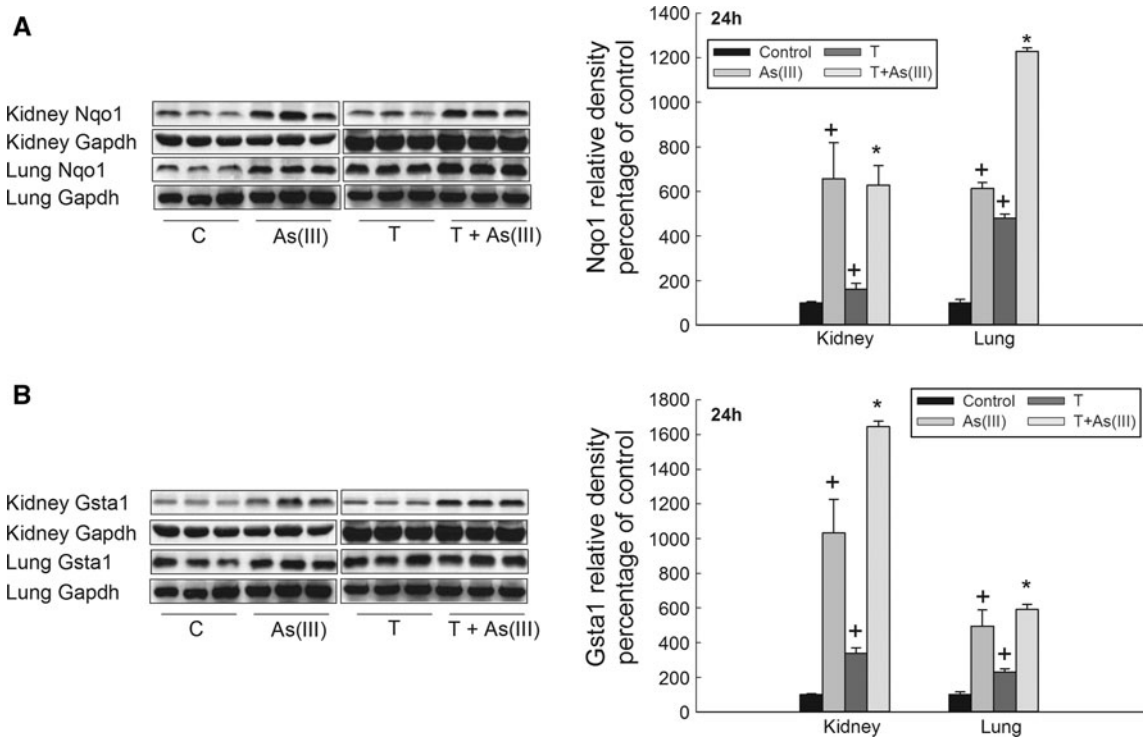


Fig. 9 Effect of co-exposure to As(III) and TCDD on Nqo1 and Gsta1 protein expression levels in the kidney and lung of C57Bl/6 mice. Kidney and lung cytosolic proteins were isolated after 24 h of treatment. 5 or 30 μg of cytosolic proteins for Nqo1 and Gsta1, respectively, was separated on a 10 % SDS-PAGE. Proteins were detected using the enhanced chemiluminescence method. The *graph* represents the relative amount of protein normalized to β-actin signals (mean ± SEM, $n = 6$), and the results are expressed as percentage of the control values taken as 100 %. $^{+}P < 0.05$, compared to control; $^{*}P < 0.05$, compared to respective TCDD treatment; $^{\wedge}P < 0.05$, compared to respective As(III) treatment

The tissue-specific expression of AhR-regulated enzymes examined in the current study and other previous studies has been a subject of discrepancies (Shimada et al. 2003; Jaiswal 2000; McMahon et al. 2001; Frericks et al. 2007). Several factors may have led to these discrepancies. The first factor is the use of a conventional PCR technique or Northern blot analyses in most of these studies, which may be insensitive because of the low expression levels of AhR-regulated genes expression, especially in extrahepatic tissues such as the heart (Shimada et al. 2003; Jaiswal 2000; McMahon et al. 2001). Secondly, most of the previous studies focused on one tissue without giving comparative information regarding the other tissues. Therefore, it was necessary to examine the expression of multiple AhR-regulated enzymes simultaneously in different organs by a sensitive technique, such as the real-time PCR technique. Our findings showed that the AhR-regulated enzymes Cyp1a1, Cyp1a2, Nqo1, and Gsta1 in addition to HO-1 are constitutively expressed in all the examined tissues. The lung had the highest constitutive expression of Cyp1a1, Cyp1a2, and Gsta1 followed by the kidney and lastly the heart. The kidney had the highest constitutive expression of Nqo1 and HO-1. Generally, the heart had the lowest level of constitutive expression of all tested genes except for Nqo1 where it came second after the kidney.

In the current study, As(III) significantly increased the Cyp1a1 mRNA in lung but not in the kidney or heart of C57Bl6 mice. Furthermore, As(III) failed to alter Cyp1a2 mRNA in all tested tissues. At the protein and catalytic activity levels, As(III) induced lung Cyp1a protein expression levels with a subsequent increase in EROD and MROD catalytic activities, while it inhibited the kidney EROD and MROD catalytic activities. The limitation of minimal protein quantities that could be extracted from heart samples hindered us from measuring protein expression and catalytic activities of AhR-regulated genes in this organ. Our results are in line with previous reports which showed that As(III) was able to induce Cyp1a1 mRNA levels in liver- and lung-derived cell lines, and lung tissues of C57BL/6 mice (Elbekai and El-Kadi 2007; Wu et al. 2009). Similarly and in agreement with our results, As(III) also failed to cause similar induction of Cyp1a1 mRNA levels in the kidney of C57Bl/6 mice (Seubert et al. 2002). We report here for the first time that As(III) did not significantly alter Cyp1a1 mRNA levels in the heart. Importantly, there has been no previous attempts to

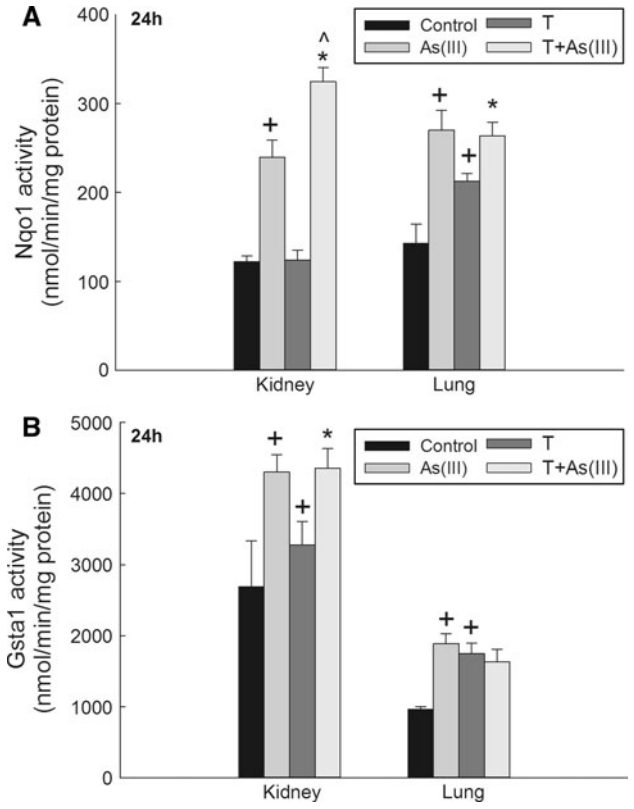


Fig. 10 Effect of co-exposure to As(III) and TCDD on Nqo1 and Gsta1 catalytic activity levels in the kidney and lung of C57Bl/6 mice. Kidney and lung cytosolic proteins were isolated after 24 h of treatment. Nqo1 enzyme activity was determined spectrophotometrically using DCPIP as substrate, and dicoumarol as specific Nqo1 inhibitor. Gsta1 activity was determined spectrophotometrically using CDNB as a substrate as described under "Materials and methods". Values are presented as mean ± SEM ($n = 6$). $^{+}P < 0.05$, compared to control; $^{*}P < 0.05$, compared to respective TCDD treatment; $^{\wedge}P < 0.05$, compared to respective As(III) treatment

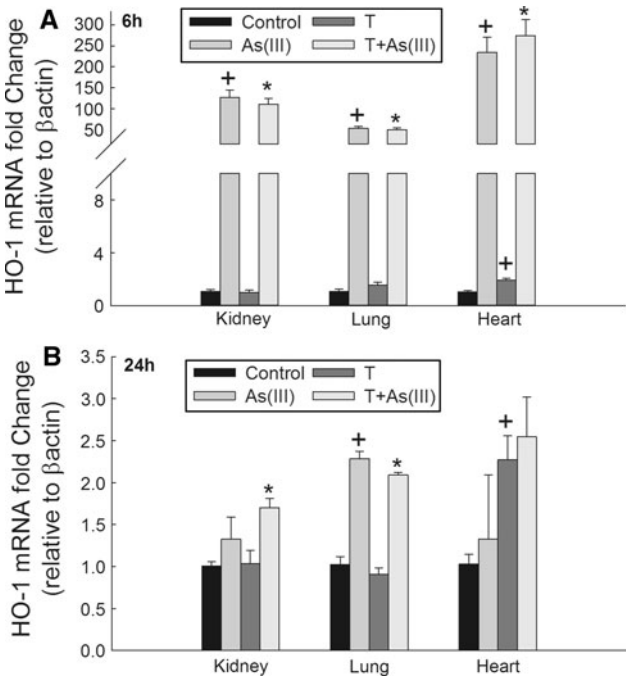


Fig. 11 Effect of co-exposure to As(III) and TCDD on HO-1 mRNA in the kidney, lung, and heart of C57Bl6 mice. Animals were injected i.p. with 12.5 mg/kg As(III) in the absence and presence of 15 μg/kg TCDD for 6 h (**a**) and 24 h (**b**). First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from kidney, and the expression of HO-1 was measured using real-time PCR. cDNA fragments were amplified and quantitated using ABI 7500 real-time PCR system as described under "Materials and methods". Duplicate reactions were performed for each experiment, and the values presented are the means of six independent experiments. $^{+}P < 0.05$, compared to control (untreated animals); $^{*}P < 0.05$, compared to respective TCDD treatment; $^{\wedge}P < 0.05$, compared to respective As(III) treatment

investigate the effects of As(III) on Cyp1a2 expression. In the current study, it was demonstrated that As(III) alters Cyp1a2 mRNA, protein, and catalytic activity levels in the kidney, lung, and heart of C57Bl/6 mice in a pattern different from that of Cyp1a1. Thus, the effect of As(III) on Cyp1a1 cannot be generalized to include Cyp1a2.

Previous reports from our laboratory have demonstrated that As(III) potentiated the TCDD-mediated induction of Cyp1a1 mRNA and protein expression levels in the mouse liver-derived cell line Hepa 1c1c7 cells after 6 h of treatment (Elbekai and El-Kadi 2007). However, it was never reported before whether As(III) would affect TCDD-mediated induction of Cyp1a1 and Cyp1a2 mRNA, protein, or catalytic activity levels in the kidney, lung, or heart of C57Bl/6 mice. Therefore, we examined the effect of As(III) on the TCDD-mediated induction of Cyp1a1 and Cyp1a2 at the mRNA, protein, and catalytic activity levels. As(III) after 6 h of treatment significantly inhibited the TCDD-mediated induction of the kidney and heart Cyp1a1 and Cyp1a2 mRNA levels, while it significantly potentiated the TCDD-mediated induction of lung Cyp1a1 but not Cyp1a2 mRNA levels. On the contrary, As(III) after 24 h of treatment significantly potentiated the TCDD-mediated induction of kidney and lung Cyp1a1 and kidney Cyp1a2 mRNA levels. At the protein and catalytic activity levels, As(III) significantly inhibited the kidney Cyp1a, while it significantly potentiated lung Cyp1a protein expression levels, which were subsequently translated to their EROD and MROD catalytic activity levels.

The differences between the results of mRNA expression obtained after 6 h and 24 h of treatment are multifactorial.

Therefore, we hypothesize that the effects observed at 6 h are the direct effect of non-metabolized As(III) in the form of sodium arsenite. However, the effect after 24 h is an indirect effect that could have been arisen due to other metabolic and kinetic changes that are summarized as following: first, the distribution and elimination half-life of As(III) from these organs are different from those of TCDD

Table 2 Summary of the effects of As(III) and TCDD on the expression of AhR-regulated genes in kidney, lung, and heart

Treatments	Genes	Kidney		Lung		Heart	
		6 h	24 h	6 h	24 h	6 h	24 h
As(III)	Cyp1a1	↔ mRNA	↔ mRNA, ↔ protein, ↓ activity	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↔ mRNA	↔ mRNA
	Cyp1a2	↔ mRNA	↔ mRNA, ↔ protein, ↓ activity	↑ mRNA	↔ mRNA, ↑ protein, ↑ activity	↔ mRNA	↔mRNA
	Nqo1	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑mRNA
	Gsta1	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑mRNA
	HO-1	↑ mRNA	↔ mRNA	↑ mRNA	↑ mRNA	↑ mRNA	↔ mRNA
TCDD	Cyp1a1	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA
	Cyp1a2	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA
	Nqo1	↑ mRNA	↑ mRNA, ↑ protein, ↔ activity	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA
	Gsta1	↑ mRNA	↑ mRNA, ↑ protein, ↑ activity	↑ mRNA	↔ mRNA, ↑ protein, ↑ activity	↑ mRNA	↑ mRNA
	HO-1	↔ mRNA	↔ mRNA	↔ mRNA	↔ mRNA	↑ mRNA	↑ mRNA
As(III) + TCDD	Cyp1a1	↓ mRNA	↑↑ mRNA, ↓ protein, ↓ activity	↑↑ mRNA	↑↑ mRNA, ↑↑ protein, ↑↑ activity	↓ mRNA	↔ mRNA
	Cyp1a2	↓ mRNA	↑↑ mRNA, ↓ protein, ↓ activity	↔ mRNA	↔ mRNA, ↑↑ protein, ↑↑ activity	↓ mRNA	↔ mRNA
	Nqo1	↑↑ mRNA	↑↑ mRNA, ↑↑ protein, ↑↑ activity	↑↑ mRNA	↑↑ mRNA, ↑↑ protein, ↑↑ activity	↑ mRNA	↑ mRNA
	Gsta1	↑↑ mRNA	↑↑ mRNA, ↑↑ protein, ↑↑ activity	↑↑ mRNA	↑↑ mRNA, ↑↑ protein, ↔ activity	↑ mRNA	↑ mRNA
	HO-1	↑↑ mRNA	↑↑ mRNA	↑↑ mRNA	↑↑ mRNA	↑ mRNA	↔ mRNA

(↑) increase; (↓) decrease; (↔) no change; (↑↑) potentiation

with TCDD possessing a longer half-life than As(III) and thus the persistent effect after 24 h of treatment (Hughes et al. 1999; Birnbaum 1986). Second, As(III) is metabolized in these organs to produce mono- and di-methyl arsenate and arsenite metabolites, which in turn will have differential effects on these enzymes than the parent compound, sodium arsenite, especially at the mRNA and protein stability levels (Cui et al. 2008). Third, there has been previous reports that have shown a biphasic effect of As(III) at different concentrations and the observed changes in the expression of AhR-regulated enzymes could be attributed to these biphasic effects (He et al. 2007). Fourth, As(III) might have affected other physiological processes or even triggered other physiological modulators such as bilirubin and biliverdin that are also AhR ligands (Denison and Nagy 2003). In the current study, we have demonstrated that As(III) alone or in the presence of TCDD was able to induce HO-1 mRNA levels as early as 6 h in all tested tissues. However, this induction was either completely abolished or reduced after 24 h of treatment. We have previously demonstrated that there is an inverse correlation between HO-1 and CYP1A1 activities (Anwar-Mohamed et al. 2009; Anwar-Mohamed and El-Kadi 2010). In this study, the correlation between HO-1 and EROD and MROD catalytic activities was proportional in the lung as As(III) potentiated the TCDD-mediated induction of Cyp1a mRNA, protein, and catalytic activity levels. This can be explained by the fact that As(III) is known to bind to hemoglobin from red blood cells causing hemolysis and increasing plasma hemoglobin levels (Klimecki and Carter 1995). Therefore, it is possible that As(III)-liberated hemoglobin might have inhibited the inhibitory effect of HO-1 on Cyp1a1 and Cyp1a2 catalytic activities in the lung. Interestingly, the main excretion route of As(III) and its metabolites is the kidney (Vahter and Concha 2001); therefore, in the kidney, the inhibitory effect of As(III) will persist as As(III) gets eliminated and plasma hemoglobin does not reach the kidney.

With regards to AhR-regulated phase II enzymes, As(III) alone significantly induced Nqo1 and Gsta1 mRNA, protein, and catalytic activities in the kidney, lung, and heart. Importantly, the co-exposure to As(III) and TCDD significantly potentiated the induction of kidney, Nqo1 mRNA after 6 h and 24 h of treatment, lung Nqo1 after 24 h of treatment, and heart Nqo1 and Gsta1 mRNA after 6 h and 24 h of treatment. As(III) is not known to be an AhR ligand, yet it has been shown to be an oxidative stress inducer (Elbekai and El-Kadi 2005). The regulation of Nqo1 and Gsta1 involves in addition to the AhR–XRE pathway and the Nrf2–antioxidant response element (ARE) pathway. However, the induction of Gsta1 mRNA levels by As(III) in the absence and presence of TCDD was much greater than the induction of Nqo1 mRNA. It is still

unknown whether mouse Gsta1 regulation involves in addition to the AhR–XRE and Nrf2–ARE pathways an additional pathway. To the contrast, potential regulatory elements have been identified in the human *GSTA1* promoter, including AP-1 and AP-2 consensus sequence and a glucocorticoid response element (Whalen and Boyer 1998). Thus, it might be a possibility that the effect of As(III) on Gsta1 might be mediated through a different pathway.

In conclusion, the present study demonstrates for the first time that As(III) modulates constitutive and TCDD-induced AhR-regulated genes in a time-, tissue-, and AhR-regulated enzyme-specific manner. Furthermore, the effect on one of these enzymes could not be generalized to other enzymes even if it is in one organ, as there are multiple factors that could interplay to cause differential effects.

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