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Modulation of cytochrome P450 1 (Cyp1) by vanadium in hepatic tissue and isolated hepatocyte of C57BL/6 mice

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

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Abstract The objective of the current study was to investigate the effect of vanadium (V^{5+}) on Cyp1 expression and activity in C57BL/6 mice liver and isolated hepatocytes. For this purpose, C57BL6 mice were injected intraperitoneally with V^{5+} (5 mg/kg) in the absence and presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (15 μg/kg) for 6 and 24 h. Furthermore, isolated hepatocytes from C57BL6 mice were treated with V^{5+} (5, 10, and 20 μM) in the absence and presence of TCDD (1 nM) for 3, 6, 12, and 24 h. In vivo, V^{5+} alone did not significantly alter Cyp1a1, Cyp1a2, or Cyp1b1 mRNA, protein, or catalytic activity levels. Upon co-exposure to V^{5+} and TCDD, V^{5+} significantly potentiated the TCDD-mediated induction of the Cyp1a1, Cyp1a2, and Cyp1b1 mRNA, protein, and catalytic activity levels at 24 h. In vitro, V^{5+} decreased the TCDD-mediated induction of Cyp1a1 mRNA, protein, and catalytic activity levels. Furthermore, V^{5+} significantly inhibited the TCDD-induced AhR-dependent luciferase activity. V^{5+} also increased serum hemoglobin (Hb) levels in animals treated for 24 h. Upon treatment of isolated hepatocytes with Hb alone or in the presence of TCDD, there was an increase in the AhR-dependent luciferase activity. When isolated hepatocytes were treated for 2 h with V^{5+} in the presence of TCDD, followed by replacement of the medium with new medium containing Hb, there was further potentiation to the TCDD-mediated effect. The present study demonstrates that there is a differential modulation of Cyp1a1 by V^{5+} in C57BL/6 mice livers and isolated hepatocytes and demonstrates Hb as an in vivo specific modulator.

Keywords Aryl hydrocarbon receptor · C57BL/6 mouse · Vanadium · Carcinogenesis

Abbreviations

AhR	Aryl hydrocarbon receptor
V^{5+}	Vanadium
Cyp450s	Cytochrome P450s
HO-1	Heme oxygenase-1
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)
XRE	Xenobiotic responsive element

Introduction

Cytochrome P450 (CYP) enzymes are a superfamily of heme-containing mono-oxygenases that catalyze the biotransformation of not only a wide range of drugs and endogenous substances, but also the bioactivation of many procarcinogens and toxins (Guengerich and Shimada 1998). Consequently, specific CYP enzymes such as CYP1A1, CYP1A2, and CYP1B1 have been identified as potential targets for cancer chemoprevention (Yang et al. 1994). The expression of the CYP1A1 gene, along with CYP1A2 and CYP1B1 genes, is regulated by the aryl hydrocarbon receptor (AhR).

The AhR is a ligand-activated cytoplasmic transcription factor that belongs to the basic-helix-loop-helix protein family (Nebert et al. 1984). In the absence of a ligand, AhR is associated with two 90-kDa heat shock protein-90 (HSP90), the 23-kDa heat shock protein (p23), and hepatitis B virus X-associated protein 2 (XAP2). Following

G. Abdelhamid · I. E. A. Amara · A. Anwar-Mohamed · 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 2E1, Canada
e-mail: aelkadi@ualberta.ca

ligand binding, AhR translocates to the nucleus, dissociates from the complex, and forms a heterodimer with the AhR nuclear translocator (ARNT) (Hankinson 1995). The whole AhR/ARNT 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. Several AhR agonists, with the exception of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are substrates for the inducible AhR-regulated CYPs enzymes. The conversion of specific AhR ligands such as the polycyclic aromatic hydrocarbons (PAHs) benzo(a)pyrene, benzo(a)anthracene, 7,12-dimethylbenz(a)anthracene, and 3-methylcholanthrene into diol epoxide compounds specifically by CYP1A1 and CYP1A2 results in the formation of covalent adducts when these genotoxic metabolites react with guanines in critical genes, which in turn lead to mutagenesis and other toxic responses (Spink et al. 2002).

The toxicological effect of individual AhR ligands is well established (Brauze et al. 2006; Germolec et al. 1996; Volotinen et al. 2009). Moreover, limited studies have examined the combined toxicological effects of these ligands with heavy metals such as vanadium (V^{5+}). V^{5+} is poorly understood trace element that became a subject of interest among scientists since the environmental contamination by V^{5+} has dramatically increased, especially in the most developed countries, due to the widespread use of fossil fuels, many of which liberate fine particulates of V^{5+} to the atmosphere during combustion (Baran 2008). Humans and other species may be exposed to V^{5+} through the atmosphere, food, and water. Foods contain V^{5+} in the concentration of 10–60 μg (Evangelou 2002). In addition, the highest level of V^{5+} supplements in multivitamin products reaches 25 μg/tablet or capsule. Furthermore, weight-training athletes are reported to use up to 18.6 mg V^{5+} per day as a body-building supplement (Barceloux 1999). An estimate of the total body pool of V^{5+} in healthy individuals is 100–200 μg (Byrne and Kosta 1978). If we take into consideration that V^{5+} is significantly deposited in kidney and liver, the concentrations used in the current study are of great relevance to those in humans.

With regard to the effect of V^{5+} on AhR-regulated genes, we have previously reported that V^{5+} was able to decrease the TCDD-mediated induction of CYP1A1 and NQO1 at mRNA, protein, and catalytic activity levels in the mouse hepatoma Hepa 1c1c7 cells (Anwar-Mohamed and El-Kadi 2008; Anwar-Mohamed and El-Kadi 2009) and in human hepatoma HepG2 cells (Abdelhamid et al. 2010a; Abdelhamid et al. 2010b). Furthermore, we have recently demonstrated that V^{5+} modulates AhR-regulated genes in vivo in C57BL/6 mice kidney, lung, and heart, in a tissue-, time-, and AhR-regulated enzyme-specific manner (Abdelhamid et al. 2012). However, to the best of our knowledge, there has been no previous attempt to examine the effect of V^{5+} on the regulation of Cyp1a1 genes in the presence and absence of TCDD in hepatic tissues in vivo and isolated hepatocytes in vitro. Therefore, we hypothesize that V^{5+} differentially alters the expression of Cyp1a1 in vivo and in vitro in C57BL/6 mice. Thus, the objective of the current study was to investigate the effect of V^{5+} in the absence and presence of TCDD on Cyp1a1 in vivo using C57BL/6 mice liver and in vitro using isolated mouse hepatocytes.

Materials and methods

Materials

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), β-glucuronidase, arylsulfatase, chlorpromazine HCL, collagenase, collagen from rat tail, 7-ethoxyresorufin, 7-methoxyresorufin, fluorescamine, hemoglobin, anti-goat IgG peroxidase secondary antibody, protease inhibitor cocktail, and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). TRIzol reagent was purchased from Invitrogen (Carlsbad, CA, USA). 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, USA). Real-time PCR primers were synthesized by Integrated DNA Technologies, Inc. 1-chloro-2,4-dinitrobenzene, 2,6-dichlorophenolindophenol, 7-ethoxyresorufin, 7-methoxy resorufin, anti-goat IgG peroxidase secondary antibody, dicoumarol, protease inhibitor cocktail, and ammonium metavanadate (NH_4VO_3) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). TCDD, >99 % pure, was purchased from Cambridge Isotope Laboratories (Woburn, MA, USA). Chemiluminescence Western blotting detection reagents were from GE Healthcare Life Sciences (Piscataway, NJ, USA). Nitrocellulose membrane was purchased from Bio-Rad Laboratories (Hercules, CA, USA). 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, USA). Antimouse IgG peroxidase secondary antibody was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). All other chemicals were purchased from Fisher Scientific (Toronto, ON, USA).

Animals and ethics

Male C57BL/6 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 toxic and a likely human carcinogen. All personnel were instructed as to safe handling procedures. Lab 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 V^{5+} (dissolved in saline) at 5 mg/kg, in the presence and absence of 15 μg/kg TCDD (TCDD was dissolved in dimethylsulfoxide (DMSO), followed by further dilution in corn oil). The mice were segregated into 4 groups. The first group ($n = 12$) was control mice received saline plus corn oil. The second group ($n = 12$) V^{5+}-treated mice received V^{5+} dissolved in saline plus corn oil. Third group ($n = 12$) TCDD-treated mice received TCDD dissolved in corn oil plus saline. The fourth group ($n = 12$) V^{5+} plus TCDD–treated mice received V^{5+} dissolved in saline plus TCDD dissolved in corn oil. Thereafter, the animals were euthanized after a single injection at 6 h ($n = 6$) or 24 h ($n = 6$) via cervical dislocation. Livers were excised, immediately frozen in liquid nitrogen, and stored at −80 °C until analysis.

Isolation of mouse hepatocytes and cell culture

Three solutions were utilized for the isolation of mouse hepatocytes as previously described (El-Kadi et al. 1997). Solution A contained (mM): NaCl 115, KCl 5, KH_2PO_4 1, HEPES 25, EGTA 0.5, glucose 5.5, and heparin 56.8 μg/ml in deionized water. Solution B was Solution A with $CaCl_2$ 1 mM, trypsin inhibitor 0.25 μM, and collagenase 0.025 %. Solution C contained 25 ml of solution B supplemented with $MgSO_4$ 1.2 mM and 75 ml of DMEM. Solutions A and B were adjusted to pH 7.4 by adding HCl 1 N and filtered through a 22-μm membrane before use.

Mouse hepatocytes were isolated by a two-step collagenase perfusion method as described previously (Seglen 1976). Mice were anaesthetized, a midline laparotomy was performed, and the portal vein was cannulated, while the suprahepatic and the inferior cava veins were cut. All tubing and solutions were maintained at 37 °C and saturated with 95 % O_2:5 % CO_2. The liver was perfused via the portal vein with 25 ml of solution A, by use of a peristaltic pump at a flow rate of 5 ml/min for 5 min, then with 35 ml of solution B at a flow rate of 5 ml/min for 7 min, until the liver appeared completely blanched and softened. The liver was maintained wet with saline during the entire period of perfusion.

After in situ perfusion, the liver was removed and placed in a petri dish containing 30 ml solution C. The capsule was stripped away from one side of the liver, and the cells were detached by brushing the liver with a plastic comb and filtered through cotton gauze. The suspension of cells was incubated in a shaker water bath at 37 °C for 5 min with 95 % O_2:5 % CO_2, filtered through a (70 μm) cell strainer and divided into two aliquots which were placed on ice. Once the temperature of 4 °C was reached, the cells were centrifuged at $100\times g$ for 2 min, the supernatant was aspirated, and the sediment was re-suspended in DMEM and re-centrifuged at $100\times g$ for 2 min, an operation that was repeated twice. The supernatant was discarded and the sediment was re-suspended in DMEM to obtain 0.5×10^6 cells/ml.

Each well of the plastic culture plates (24-well, Falcon, Becton–Dickinson Labware, NJ, USA) was coated with 500 μl of Type I rat-tail collagen (50 μg/ml acetic acid 0.02 N) overnight, the wells were rinsed with deionized water and washed once with serum-free DMEM before the hepatocytes ($0.25 \times 10^6 \times 500\ \mu l^{-1}$ per well) were added in DMEM supplemented with 10 % fetal bovine serum, 1 μM insulin, 50 ng/ml dexamethasone phosphate, 100 IU/ml penicillin G, 10 μg/ml streptomycin, and 25 ng/ml amphotericin B. The plastic culture plates were incubated at 37 °C in a cell culture incubator with 5 % CO_2. Viability was assessed before and after the incubation period by the trypan blue (0.2 %) exclusion method, and in both instances the viability was over 90 %.

Chemical treatments

Cells were treated in serum-free medium with V^{5+} (5, 10, and 20 μM) in the absence and presence of TCDD (1 nM) and/or Hb (1 μM) as described in figure legends. V^{5+} (10 mM) stock was prepared freshly in double de-ionized water. TCDD was dissolved in DMSO and maintained in DMSO at −20 °C until use. Hb was dissolved in DMEM and maintained in DMEM at −20 °C until use. In all treatments, the DMSO concentration did not exceed 0.01 % (v/v).

Effect on cell viability

The effect of V^{5+} on cell viability was determined using the MTT assay as described previously (Mosmann 1983). MTT assay measures the conversion of MTT to formazan in living cells via mitochondrial enzymes of viable cells. In

brief, hepatocytes 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 V^{5+} (5–100 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, USA).

RNA extraction and cDNA synthesis

Total RNA from the frozen tissues or treated cells for the specific time periods 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 by 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 for 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), 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 the current study are listed in Table 1. 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 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 glyceraldehyde-3-phosphate dehydrogenase (Gapdh) 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 (untreated control) 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.

Table 1 Primers sequences and efficiency for real-time PCR reactions

Gene	Forward primer	Reverse primer	Slope	Amplification	Efficiency
B-actin	5′-TAT TGG CAA CGA GCG GTT CC-3′	5′-GGC ATA GAG GTC TTT ACG GAT GTC-3′	−3.2799	2.017843	1.017843
Cyp1a1	5′-GGT TAA CCA TGA CCG GGA ACT-3′	5′-TGC CCA AAC CAA AGA GAG TGA-3′	−3.0811	2.017843	1.111346
Cyp1a2	5′-TGG AGC TGG CTT TGA CAC AG-3′	5′-CGT TAG GCC ATG TCA CAA GTA GC-3′	−3.7134	1.859067	0.859067
Cyp1b1	5′-AAT GAG GAG TTC GGG CGC ACA-3′	5′-GGC GTG TGG AAT GGT GAC AGG-3′	−3.1697	2.067699	1.067699
HO-1	5′-GTG ATG GAG CGT CCA CAG C-3′	5′-TGG TGG CCT CCT TCA AGG-3′	−3.6159	1.890411	0.890411

Efficiency values were measured using the CT slope method. This method involves generating a dilution series of the target template and determining the CT value for each dilution. A plot of CT versus log cDNA concentration range (with four concentrations points at 20, 2, 0.2, and 0.02 ng/μl) is constructed. Exponential amplification was calculated using the equation: $A = 10^{(-1/\text{slope})}$. Efficiency was calculated using the equation: $E = 10^{(-1/\text{slope})-1}$

Preparation of microsomal protein fractions

Tissue-specific microsomes were prepared using a previously published method (Barakat et al. 2001). Briefly, liver tissues were washed in ice-cold KCl (1.15 %, KCl w/v), cut into pieces, and homogenized separately in ice-cold sucrose solution (1 g of tissue in 25 ml of 0.25 M sucrose). Tissue homogenates were centrifuged at 600×*g* for 8 min. The supernatant was then centrifuged at 12,000×*g* for 10 min. Thereafter, supernatants resulting from the previous step were mixed with 8 mM CaCl2 and centrifuged at 27,000×*g* for 15 min. Calcium precipitation eliminates the need for ultracentrifugation, but may result in the degradation of some proteins by calcium-activated proteases. Thus, caution should be exercised when preparing microsomes using this method. The consequential pellets were suspended in 0.15 M KCl and re-centrifuged at 27,000×*g* for 15 min. Final microsomal pellets were re-suspended in cold sucrose and were stored at −80 °C. Thereafter, microsomal protein concentrations were determined by the Lowry method using bovine serum albumin as a standard (Lowry et al. 1951).

Protein extraction from hepatocytes

Twenty-four hours after incubation with the test compounds, cells were collected in lysis buffer containing 50 mM HEPES, 0.5 M NaCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 10 % (v/v) glycerol, 1 % Triton X-100, and 5 µl/ml of protease inhibitor cocktail. The cell homogenates were obtained by incubating the cell lysates on ice for 1 h, with intermittent vortex every 10 min, followed by centrifugation at 12,000×*g* for 10 min at 4 °C. The supernatant total cellular lysate was collected and stored at −80 °C.

Western blot analysis

Western blot analysis was performed using a previously described method (Sambrook et al. 1989). Briefly, 20 µg of liver microsomal proteins or total cell lysates were 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 NaCl, 3 mM KCl, 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 mouse monoclonal Cyp1a1/1a2, primary rabbit polyclonal Cyp1b1, or primary polyclonal rabbit anti-mouse actin for 2 h at room temperature. Incubation with a peroxidase-conjugated goat anti-rabbit IgG secondary antibody for Cyp1b1 and actin or goat anti-mouse IgG secondary antibody for Cyp1a1/1a2 was carried out for another 1 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, USA). The intensity of the protein bands were quantified, relative to the signals obtained for actin, using ImageJ software [National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij].

Determination of Cyp1a1 enzymatic activity in hepatocytes

A published EROD activity assay in 96-well plates (Vakharia et al. 2001) was used with modifications. The culture medium in the wells was replaced with 100 µl/well of culture medium containing 8 µM 7-ethoxyresorufin and dicoumarol (10 µM) (NAD(P)H-quinone oxidoreductase inhibitor). After 45-min incubation at 37 °C in a CO2 incubator, 75 µl of culture media from each well was transferred to white, opaque 96-well plates. Thereafter, 15 µl of 1:100 Glucuronidase/sulfatase was added to each well. The plate was incubated for 2 h at 37 °C, and then, the fluorescence of each well was measured using Baxter 96-well fluorescence plate reader with 545-nm excitation and 575-nm emission filters. Enzymatic activity was normalized for cellular protein content, which was determined using a modified fluorescent assay (Lorenzen and Kennedy 1993).

Microsomal incubation and measuring Cyp1a1 catalytic activity

EROD and MROD activities were performed on subcellular fraction, as previously described (Pohl and Fouts 1980). Microsomes from liver 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 of 7-ethoxyresorufin or 7-methoxyresorufin for Cyp1a1 and Cyp1a2, respectively. 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.

Transient transfection of hepatocytes and luciferase assay

Hepatocytes were plated onto 12-well cell culture plates. Each well of cells was transfected with 1.6 μg of XRE-driven luciferase reporter plasmid pGudLuc1.1, generously provided by Dr. M. S. Denison (University of California, Davies), and 0.1 μg of the renilla luciferase pRL-CMV vector, used for normalization, using lipofectamine 2,000 reagent according to manufacturer's instructions (Invitrogen). Luciferase assay was performed according to manufacturer's instructions (Promega). In brief, after incubation with test compounds for 24 h, cells were washed with PBS, 250 μl of 1 × passive lysis buffer was added into each well with continuous shaking for at least 15 min, and then the content of each well was collected separately in 1.5-ml microcentrifuge tubes. Enzyme activities were determined using a Dual-Luciferase reporter assay system (Promega). Quantification was performed using a TD-20/20 luminometer (Turner BioSystems, Sunnyvale, CA, USA).

Measuring serum Hb levels

Measurement of serum Hb levels was carried out as previously described (Vazquez et al. 1991). In brief, stock solution of Hb was prepared (1 mg/ml) and the exact Hb content was determined by the Hartree method (Hartree 1972). The reaction mixture was prepared by the addition of the following solutions to a test tube, in the following order: 1.5 ml of 1 % NaCl, 1 ml of glacial acetic acid, 0.01 ml of 0.25 M EDTA solution, 0.2 ml of 0.5 M chlorpromazine HCL solution, and 0.3 ml of 6 % H_2O_2. After careful mixing, the solution was preincubated for 5 min at room temperature. Thereafter, 10 μl of serum or Hb standard solution were added and the reaction was allowed to continue for about 1 min. Absorbance was read at 1 min intervals against water at 525 nm.

Statistical analysis

Data are presented as mean ± standard error of the mean. Control and treatment measurements were compared using a one-way analysis of variance followed by a Student–Newman–Keuls post hoc comparison. A result was considered statistically significant where $P < 0.05$.

Results

Effect of co-exposure to V^{5+} and TCDD on Cyp1a1, Cyp1a2, and Cyp1b1 mRNA level in the liver of C57BL/6 mice

V^{5+} alone did not cause any significant changes in Cyp1a1, Cyp1a2, and Cyp1b1 mRNA levels at 6 and 24 h (Fig. 1a–c). Treatment with TCDD alone at 6 h significantly induced the Cyp1a1, Cyp1a2, and Cyp1b1 mRNA levels by 6,698-, 11-, and 4-fold, respectively. Similarly, treatment with TCDD for 24 h caused a significant induction of Cyp1a1, Cyp1a2, and Cyp1b1 gene expression by 6,221-, 24-, and 1,615-fold, respectively, compared to control (Fig. 1a–c). Upon co-exposure to V^{5+} and TCDD, V^{5+} at

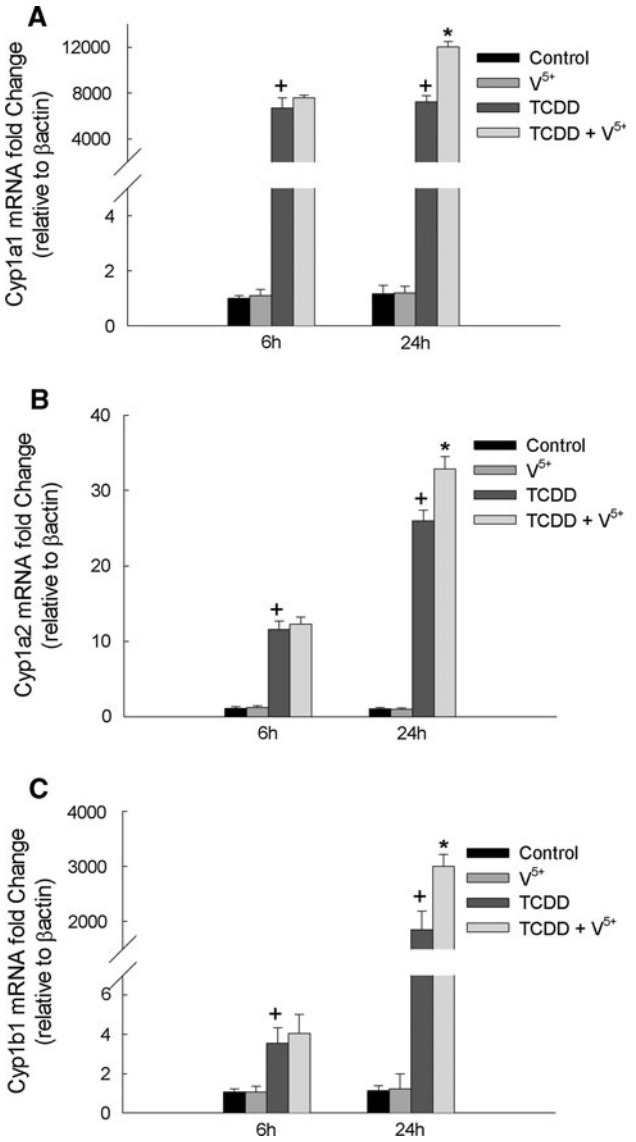


Fig. 1 Effect of co-exposure to V^{5+} and TCDD on liver Cyp1a1, Cyp1a2, and Cyp1b1 mRNA in C57BL/6 mice. Animals were injected i.p. with 5 mg/kg V^{5+} in the absence and presence of 15 μg/kg TCDD for 6 and 24 h. First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from liver, and the expression of Cyp1a1, Cyp1a2, and Cyp1b1 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 as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control (untreated animals); (*) $P < 0.05$, compared to respective TCDD treatment

6 h did not cause any significant change in TCDD-mediated induction of Cyp1a1, Cyp1a2, or Cyp1b1 at the mRNA levels in the liver. While, V^{5+} at 24 h significantly potentiated the Cyp1a1, Cyp1a2, and Cyp1b1 mRNA levels by 0.66-, 0.37- and 0.63-fold, respectively, compared to TCDD alone (Fig. 1a–c).

Effect of co-exposure to V^{5+} and TCDD on Cyp1a and Cyp1b1 protein expression and catalytic activities levels in the liver of C57BL/6 mice

Our results showed that in the presence of V^{5+} alone, no changes were observed in the Cyp1a and Cyp1b1 at the protein and catalytic activity levels. On the other hand, TCDD alone significantly induced the Cyp1a and Cyp1b1 protein expression levels to 10- and 6-fold, respectively (Fig. 2a). when animals were co-exposed to V^{5+} and TCDD, V^{5+} significantly potentiated the TCDD-mediated induction of the Cyp1a and Cyp1b1 protein expression levels by 0.79- and 0.33-fold, respectively, compared to TCDD alone (Fig. 2a).

At the catalytic activity levels, V^{5+} alone did not significantly affect the EROD or MROD activity (Fig. 2b, c). TCDD alone significantly induced EROD and MROD activities to 8- and 6-fold, respectively, compared to control. When animals were co-exposed to V^{5+} and TCDD, V^{5+} significantly potentiated the TCDD-mediated induction of EROD and MROD catalytic activities to 0.8-, and 0.72-fold, respectively, compared to TCDD alone (Fig. 2b, c).

Effect of co-exposure to V^{5+} and TCDD on HO-1 mRNA levels in the liver of C57BL/6 mice

V^{5+} alone at 6 and 24 h did not affect HO-1 mRNA levels (Fig. 3). While, TCDD alone significantly induced HO-1 mRNA levels at 6 h by 4.1-fold, compared to control. Whereas at 24 h, TCCD did not significantly affect HO-1 mRNA levels (Fig. 3). When animals were co-exposed to V^{5+} and TCDD, V^{5+} at 6 h significantly potentiated the TCDD-mediated induction of HO-1 mRNA level in the liver by 1.03-fold, compared to TCDD alone. On the other hand, at 24 h, V^{5+} did not significantly affect HO-1 mRNA levels (Fig. 3).

Effect of co-exposure to V^{5+} and TCDD on cell viability

To determine the non-toxic concentrations of V^{5+} to be utilized in the current study, isolated mouse hepatocytes were exposed for 24 h with increasing concentrations of V^{5+} (5–100 μM) in the absence and presence of 1 nM

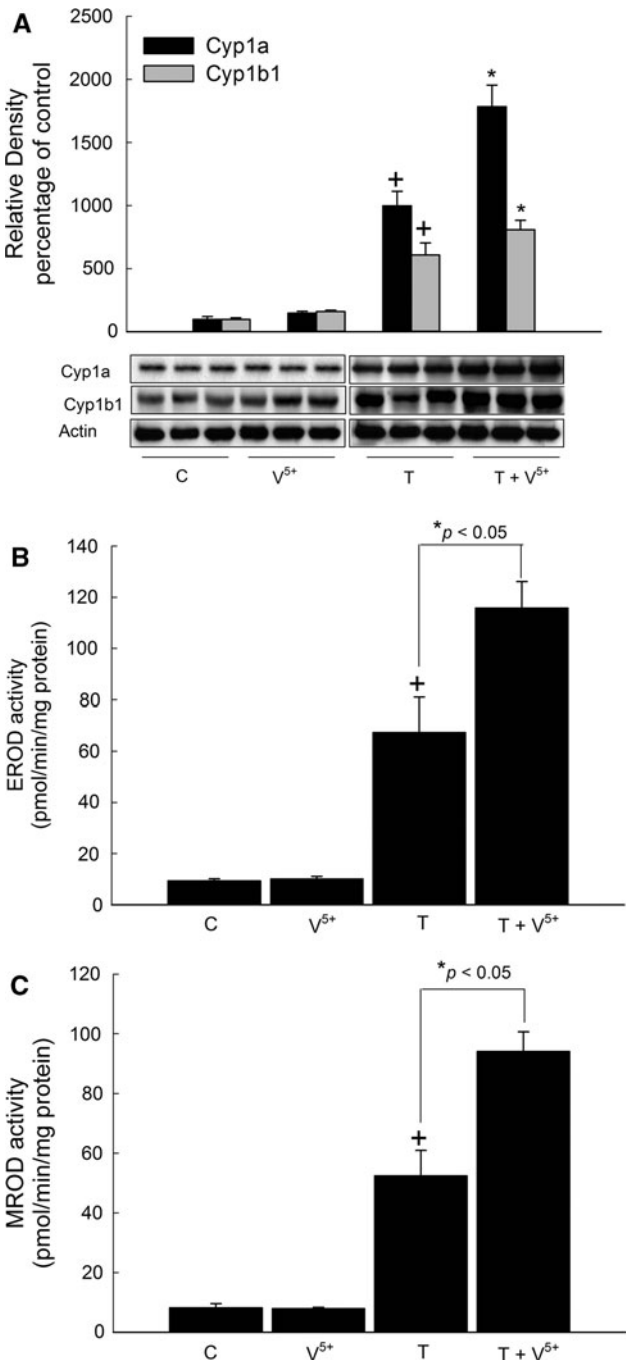


Fig. 2 Effect of co-exposure to V^{5+} and TCDD on Cyp1a and Cyp1b1 protein expression and catalytic activities levels in the Liver of C57BL/6 mice. **a** Liver microsomal proteins were isolated after 24 h of treatment. Thirty micro gram of microsomal proteins were separated on a 10 % SDS-PAGE. Proteins were detected using the enhanced chemiluminescence method. The graph represents the relative amount of protein normalized to actin signals (mean ± SE, $n = 6$), and the results are expressed as percentage of the control values taken as 100 %. **b**, **c** 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 methanol. Values are presented as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control; (*) $P < 0.05$, compared to respective TCDD treatment

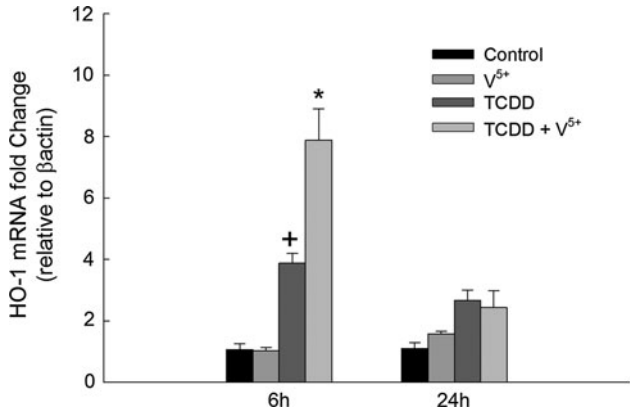


Fig. 3 Effect of co-exposure to V^{5+} and TCDD on liver HO-1 mRNA in C57BL/6 mice. Animals were injected i.p. with 5 mg/kg V^{5+} in the absence and presence of 15 μg/kg TCDD for 6 and 24 h. First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from liver, and the expression of HO-1 was 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 as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control (untreated animals); (*) $P < 0.05$, compared to respective TCDD treatment

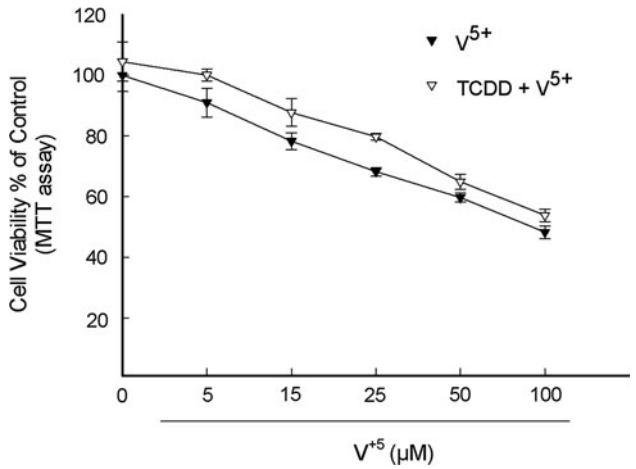


Fig. 4 Effect of V^{5+} on cell viability. Isolated mouse hepatocytes cells were treated for 24 h with V^{5+} (0, 5, 10, 20, 50 and 100 μM) in the absence and presence of 1 nM TCDD. Cell cytotoxicity was determined using MTT assay. Data are expressed as percentage of untreated control (which is set at 100 %) ± SE ($n = 8$). (+) $P < 0.05$, compared to control (concentration = 0 μM); (*) $P < 0.05$, compared to respective TCDD treatment

TCDD; thereafter, cytotoxicity was assessed using MTT assay. Figure 4 shows that V^{5+} at concentrations of 5–20 μM in the presence and absence of 1 nM TCDD did not affect cell viability. Therefore, all subsequent studies were conducted using the concentration range of 5–20 μM.

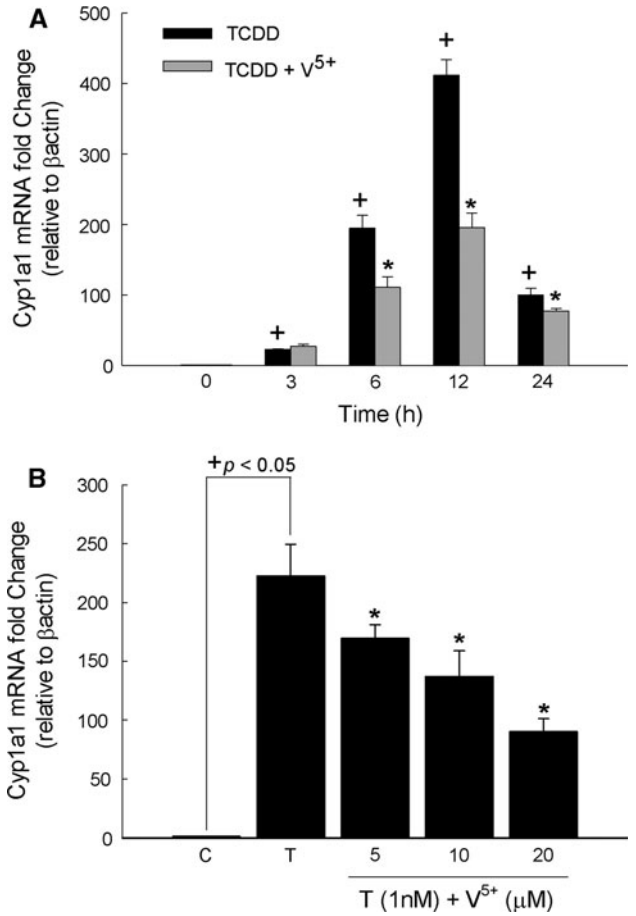


Fig. 5 Time- and concentration-dependent effect of V^{5+} on Cyp1a1 mRNA in isolated mouse hepatocytes. Hepatocytes were treated for different time points for time-dependent (**a**) or with increasing concentrations of V^{5+} in the presence of 1 nM TCDD for 6 h for concentration-dependent (**b**). First-strand cDNA was synthesized from total RNA (1.5 μg) extracted from isolated mouse hepatocytes. 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 as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control (C) (concentration = 0 μM or time = 0 h); (*) $P < 0.05$, compared to respective TCDD (T) treatment

Time- and concentration-dependent effects of co-exposure to V^{5+} and TCDD on Cyp1a1 mRNA levels in isolated mouse hepatocytes

To better understand the kinetics of Cyp1a1 mRNA in response to the co-exposure to V^{5+} and TCDD, the time-dependent effect was determined at various time points up to 24 h after treatment of isolated mouse hepatocytes with 1 nM TCDD in the absence and presence of 10 μM V^{5+}. Figure 5a shows that TCDD induced Cyp1a1 mRNA in a time-dependent manner. TCDD treatment caused a maximal induction of the Cyp1a1 mRNA by 411-fold at 12 h, compared to 0 h (Fig. 5a). In contrast, when isolated mouse hepatocytes were co-exposed to V^{5+} and TCDD,

there was a significant inhibition in the Cyp1a1 mRNA levels that occurred as early as 6 h by −0.43-fold, compared to TCDD alone (Fig. 5a).

To examine the effect of co-exposure to V^{5+} and TCDD on Cyp1a1 mRNA, isolated mouse hepatocytes were treated with various concentrations of V^{5+} in the presence of 1 nM TCDD (Fig. 5b). Thereafter, Cyp1a1 mRNA was assessed using real-time PCR. TCDD alone caused 212-fold increase in Cyp1a1 mRNA levels that was inhibited in a dose-dependent manner by V^{5+}. Initially, V^{5+} at the concentration of 5 μM caused a significant inhibition to the TCDD-mediated induction of Cyp1a1 mRNA levels by −0.24-fold. The maximum inhibition took place at the highest concentration tested, 20 μM, which caused a significant inhibition to the TCDD-mediated induction of Cyp1a1 mRNA levels by −0.6-fold, compared to TCDD alone (Fig. 5b).

Concentration-dependent effect of co-exposure to V^{5+} and TCDD on Cyp1a protein and catalytic activity in isolated mouse hepatocytes

To examine whether the observed inhibition of the TCDD-mediated induction of Cyp1a1 mRNA by V^{5+} is further translated to the protein and activity levels, isolated mouse hepatocytes were treated for 24 h with increasing concentrations of V^{5+} in the presence of 1 nM TCDD. Figure 6a, b show that TCDD alone caused 3- and 21-fold increase in Cyp1a protein and EROD catalytic activity, respectively. Of interest, V^{5+} decreased the TCDD-mediated induction of Cyp1a protein and EROD catalytic activity levels in a dose-dependent manner. This inhibitory effect of V^{5+} on the TCDD-mediated induction of Cyp1a protein and subsequently EROD catalytic activity levels is in concordance with the observed effect at the mRNA levels, in which the initial significant inhibition took place at 5 μM V^{5+} and reached the maximal inhibition at 20 μM (Fig. 6a, b).

Transcriptional inhibition of Cyp1a by V^{5+}

Isolated mouse hepatocytes were transiently transfected with the XRE-driven luciferase reporter gene in order to study the effect of V^{5+} on the AhR-dependent transcriptional activation. Luciferase activity results showed that 10 μM V^{5+} alone did not affect the constitutive expression of the luciferase activity (Fig. 7). On the other hand, 1 nM TCDD alone caused a significant increase of luciferase activity by 12.75-fold as compared to control (Fig. 7). Interestingly, co-treatment with V^{5+} and TCDD significantly inhibited the TCDD-mediated induction of luciferase activity by −0.45-fold, compared to TCDD alone (Fig. 7).

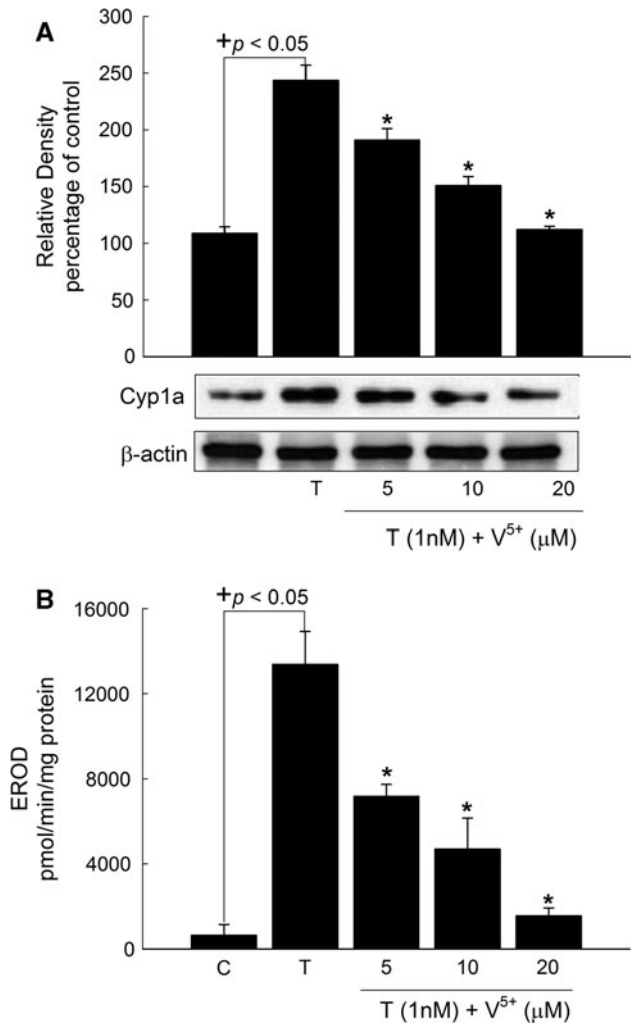


Fig. 6 Effect of V^{5+} on Cyp1a protein and catalytic activity in isolated mouse hepatocytes. Hepatocytes were treated with increasing concentrations of V^{5+} in the presence of 1 nM TCDD for 24 h for protein and catalytic activity. **a** Protein (20 μg) was separated on a 10 % SDS-PAGE and transferred to nitrocellulose membrane. Protein blots were then blocked overnight at 4 °C and then incubated with a primary Cyp1a antibody for 24 h at 4 °C, followed by 1 h incubation with secondary antibody at room temperature. Cyp1a protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to actin signals, which was used as loading control. One of three representative experiments is shown. **b** Cyp1a1 activity was measured in intact living cells treated with increasing concentrations of V^{5+}, in the absence and presence of 1 nM TCDD for 24 h. Cyp1a1 activity was measured using 7-ethoxyresorufin as a substrate. Values are presented as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control (C); (*) $P < 0.05$, compared to respective TCDD (T) treatment

The effect of V^{5+} on serum Hb level in vivo and the effect of Hb on XRE-luciferase activity in vitro

The discrepancy between the effects of V^{5+} on the TCDD-mediated induction of Cyp1a1 mRNA, protein, and catalytic activity levels in vivo and those observed at the

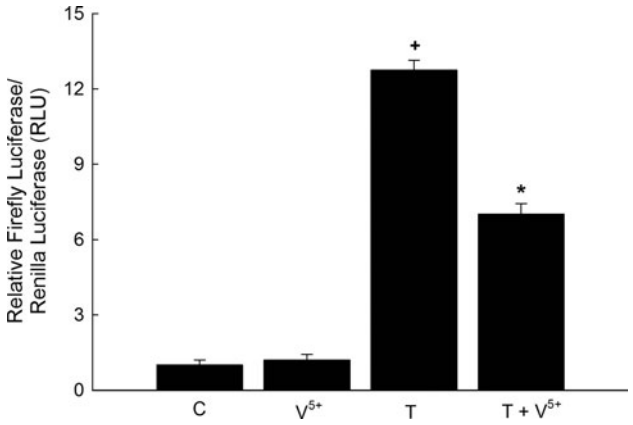


Fig. 7 Effect of V^{5+} on luciferase activity in isolated mouse hepatocytes. Hepatocytes were transiently transfected with the XRE-luciferase transporter plasmid pGudLuc1.1 and renilla luciferase plasmid pRL-CMV plasmid. Cells were treated with vehicle, V^{5+} (10 μM), TCDD (1 nM), TCDD (1 nM) + V^{5+} (10 μM) for 24 h. Cells were lysed and luciferase activity was measured according to manufacturer's instruction. Luciferase activity is reported relative to renilla activity. Values are presented as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control (C); (*) $P < 0.05$, compared to respective TCDD (T) treatment

in vitro level prompted us to examine the role of an endogenous mediator that could have influenced the effect of V^{5+} on the TCDD-mediated induction of Cyp1a1 in vivo but not in vitro. For this purpose, and given the fact that V^{5+} is a potent hemolysis–inducing agent (Hansen et al. 1986; Hogan 1990), we measured serum Hb levels in C57BL/6 mice treated with V^{5+} in the absence and presence of TCDD. Our results demonstrated that V^{5+} increased serum Hb levels by 1.49-fold, compared to control (Fig. 8a). On the other hand, TCDD alone did not significantly affect serum Hb levels. Interestingly, when animals were co-exposed to V^{5+} and TCDD, V^{5+} increased serum Hb levels by 1.75-fold, compared to TCDD alone (Fig. 8a).

The results of V^{5+} on serum Hb levels further motivated us to examine its effect alone on the XRE-driven luciferase activity. For this purpose, isolated mouse hepatocytes were treated with 1 μM Hb in the absence and presence of 1 nM TCDD. Our results demonstrated that Hb alone was able to significantly induce luciferase activity by 3.7-fold, compared to control (Fig. 8b). Moreover, TCDD alone significantly induce luciferase activity by 43.99-fold, compared to control. Interestingly, when isolated mouse hepatocytes were co-exposed to Hb and TCDD, Hb significantly potentiated the TCDD-mediated induction of luciferase activity by 1.39-fold, compared to TCDD alone (Fig. 8b).

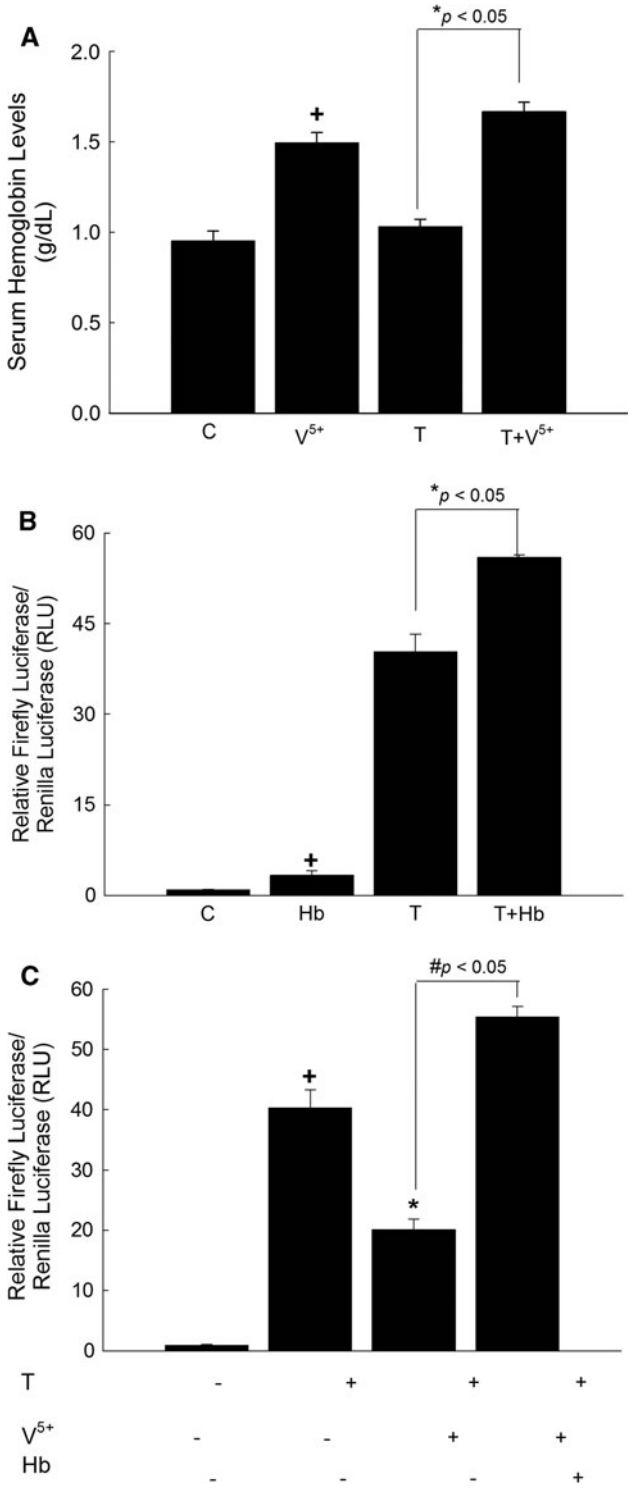
In order to correlate the in vivo effects with those observed at the in vitro levels, we tried to mimic the in vivo situation using an in vitro model. For this purpose, isolated mouse hepatocytes were treated with TCDD in the absence and the presence of V^{5+}. After 2 h, the medium was replaced with new medium for vehicle-treated cells, and for cells that were treated with V^{5+} and TCDD, the medium was replaced with new medium containing 1 μM Hb and TCDD. The reason behind replacing the medium with new treatment medium is that we needed to remove V^{5+} from the medium to rule out any direct effect of V^{5+}. Interestingly, our results have shown that replacement of V^{5+} with Hb reversed the inhibitory effect of V^{5+} which turned out to be significantly higher than the TCDD-mediated induction of XRE-driven luciferase activity by 1.37-fold (Fig. 8c).

Discussion

Humans and other types of life may be exposed to V^{5+} through the atmosphere, food, and water. As such, food contains V^{5+} in the order of 10–60 μg (Evangelou 2002). Furthermore, the level of V^{5+} in multivitamin supplements reaches 25 μg/tablet or capsule. In addition, weight-training athletes use up to 18.6 mg/day V^{5+} as a body-building supplement (Barceloux 1999). An estimate of the total body burden of V^{5+} in healthy individuals ranges from 100 to 200 μg (Byrne and Kosta 1978). Rain samples from rural and urban sites in North America and Europe had V^{5+} concentrations ranging from 1.1 to 46 μg/L (Galloway et al. 1982). Bearing in mind these concentrations, Mamane and Pirrone (1998) calculated total deposition rates of V^{5+} to be 0.1–10 kg/ha/year for urban sites, and 0.01–0.1 kg/ha/year for rural sites (Mamane and Pirrone 1998). If we take into consideration the fact that heavy metals such as V^{5+} are significantly deposited in hepatocytes (Setyawati et al. 1998; Talvitie and Wagner 1954), the concentrations used in the current study are of great relevance to those in humans. Importantly, the concentrations of V^{5+} and TCDD utilized in the current study were selected based on previous studies (Beyhl and Mayer 1983; Dafnis et al. 1992; Roman et al. 1981; Uno et al. 2008).

In the current study, we have demonstrated that V^{5+} differentially modulates the TCDD-mediated induction of Cyp1a in C57BL/6 mouse liver and isolated hepatocytes. This organ was selected as previous studies have demonstrated that V^{5+} was able to decrease the TCDD-mediated induction of Cyp1a1 at mRNA, protein, and catalytic activity levels in the mouse hepatoma Hepa 1c1c7 cells and human hepatoma HepG2 cells (Abdelhamid et al. 2010a; Anwar-Mohamed and El-Kadi 2008). Accordingly, there was a necessity to understand the consequence upon the co-exposure to TCDD and V^{5+} on Cyp1a1 at the in vivo level and if we can correlate our in vitro results to those obtained in vivo.

There is no previous study that examined the effect of V^{5+} on the constitutive and TCDD-mediated induction of



◀ **Fig. 8** Effect of V^{5+} on serum Hb levels in vivo and the effect of Hb on luciferase activity in isolated mouse hepatocytes. **a** Animals were injected i.p. with 5 mg/kg V^{5+} in the absence and presence of 15 μg/kg TCDD for 24 h. Total blood was collected and centrifuged to isolate serum. Serum Hb levels were measured as previously described under Materials and methods section. **b** Hepatocytes were transiently transfected with the XRE-luciferase transporter plasmid pGudLuc1.1 and renilla luciferase plasmid pRL-CMV plasmid. Cells were treated with vehicle, Hb (1 μM), TCDD (1 nM), TCDD (1 nM) + Hb (1 μM) for 24 h. Cells were lysed and luciferase activity was measured according to manufacturer's instruction. Luciferase activity is reported relative to renilla activity. Values are presented as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control (C); (*) $P < 0.05$, compared to respective TCDD (T) treatment. **c** Hepatocytes were transiently transfected with the XRE-luciferase transporter plasmid pGudLuc1.1 and renilla luciferase plasmid pRL-CMV plasmid. Cells were treated with vehicle, TCDD (1 nM), TCDD (1 nM) + V^{5+} (10 μM). After 2 h, the medium containing V^{5+} and TCDD was replaced with other medium containing 1 μM Hb and TCDD. Cells were lysed and luciferase activity was measured according to manufacturer's instruction. Luciferase activity is reported relative to renilla activity. Values are presented as mean ± SE ($n = 6$). (+) $P < 0.05$, compared to control (C); (*) $P < 0.05$, compared to respective TCDD (T) treatment

Cyp1a1, Cyp1a2, or Cyp1b1 mRNA or protein expression levels in vivo. In the current work, V^{5+} alone did not significantly change the mRNA, protein, or catalytic activity levels of all genes tested at both time points investigated. Furthermore, treatment of the animals with TCDD alone significantly increased the Cyp1a1, Cyp1a2, and Cyp1b1 mRNA, protein, and catalytic activity levels at 6 and 24 h.

Previous reports from our laboratory have demonstrated that V^{5+} was able to decrease the TCDD-mediated induction of Cyp1a1 at the mRNA, protein, and catalytic activity levels in the mouse hepatoma Hepa 1c1c7 cells (Anwar-Mohamed and El-Kadi 2008) and in human hepatoma HepG2 cells (Abdelhamid et al. 2010a). However, it was not reported previously whether V^{5+} could affect the TCDD-mediated induction of Cyp1a1, Cyp1a2, or Cyp1b1 mRNA, protein, or catalytic activity levels in the liver of C57BL/6 mice. Therefore, in the present study, we examined the effect of V^{5+} on the TCDD-mediated induction of Cyp1a1, Cyp1a2, and Cyp1b1 at the mRNA, protein, and catalytic activity levels. Our results showed that V^{5+} after 6 h of treatment did not alter the TCDD-mediated induction of Cyp1a1, Cyp1a2, or Cyp1b1 at the mRNA levels, while it significantly potentiated their mRNA levels at 24 h.

The differences observed between the effect of V^{5+} on the TCDD-mediated induction of Cyp1a1, Cyp1a2, and Cyp1b1 mRNA at 6 h and 24 h time points might be due to multiple factors. First, the kinetic factor in which it was previously shown that the distribution and elimination half-life of V^{5+} from the liver is different from those of TCDD, with TCDD possessing a longer half-life than V^{5+}. Thus, we hypothesize that the effects observed at 6 h are due to the direct effect of V^{5+}. However, the effect after 24 h is an indirect effect. Second, V^{5+} might have affected other physiological processes or even triggered the release of other physiological modulators such as plasma hemoglobin

levels, and these in turn modulate Cyp1a1 (Klimecki and Carter 1995).

To test our hypothesis that the effect of V^{5+} on the TCDD-mediated induction of Cyp1a mRNA levels at 24 h are in fact due to the indirect effect of V^{5+}, we tried to measure V^{5+} levels in serum of animals treated for 24 h utilizing the slow poke reactor. Interestingly, we could not detect V^{5+} in the serum of animals treated for 24 h (data not shown). In line with these findings, we found that V^{5+} in the presence of TCDD was able to induce liver HO-1 mRNA levels as early as 6 h. However, this induction was completely abolished after 24 h of treatment.

When we treated isolated mouse hepatocytes with increasing concentration of V^{5+} in the presence of TCDD, V^{5+} decreased Cyp1a1 at the mRNA, protein, and catalytic activity levels in a time- and dose-dependent manner. In agreement with these findings, previous reports from our laboratory have demonstrated that V^{5+} inhibited the TCDD-mediated induction of Cyp1a1 at the mRNA, protein, and catalytic activity levels in the C57BL/6 mouse liver–derived Hepa 1c1c7 cells (Anwar-Mohamed and El-Kadi 2008). The transcriptional regulation of *Cyp1a1* gene expression by V^{5+} was also investigated. Our results demonstrated that V^{5+} in the presence of TCDD was able to significantly decrease the AhR-dependent XRE-driven luciferase reporter activity. In agreement with these findings, we have previously shown that V^{5+} was able to decrease the AhR-dependent XRE-driven luciferase reporter activity in Hepa 1c1c7 cells (Anwar-Mohamed and El-Kadi 2008).

The fact that V^{5+} increases hemolysis and causing liberation of Hb to the circulating serum prompted us to measure serum Hb levels from animals treated with V^{5+} for 24 h (Amoyal et al. 2007; Obianime et al. 2009). Our results demonstrated that V^{5+} in the absence and presence of TCDD increased serum Hb levels. In this regard, it has been previously demonstrated that Hb is capable of increasing CYP1A1 activity in human HepG2 cells (Anwar-Mohamed and El-Kadi 2010). Furthermore, we have recently demonstrated that Hb through an AhR-dependent mechanism increases the XRE-driven luciferase activity with a subsequent increase in Cyp1a protein expression levels in C57BL/6 mouse–isolated hepatocytes (Amara et al. 2012). Therefore, we hypothesized that if we replaced the medium containing V^{5+} (direct effect) with another medium containing Hb (indirect effect) after 2 h, this will mimic the in vivo situation. Interestingly, our results have shown that replacement of V^{5+} with Hb reversed the inhibitory effect of V^{5+} on Cyp1a1. The results demonstrated that the in vivo effect of V^{5+} on the TCDD-mediated induction of Cyp1a1 mRNA, protein, and catalytic activity levels at 24 h could be attributed to the effect of Hb on the AhR signaling rather than the direct effect of V^{5+}.

In conclusion, the present study demonstrates for the first time that V^{5+} differentially modulates the TCDD-mediated induction of Cyp1a1 mRNA, protein, and catalytic activity in C57BL/6 mouse liver and isolated hepatocytes. Furthermore, the in vivo effect of V^{5+} could not be simply extrapolated from in vitro studies as there are several factors that can complicate the in vivo results which are not present at the in vitro level.

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Conflict of interest The authors declare no conflict of interest.

References

Abdelhamid G, Anwar-Mohamed A, Badary OA, Moustafa AA, El-Kadi AO (2010a) Transcriptional and posttranscriptional regulation of CYP1A1 by vanadium in human hepatoma HepG2 cells. Cell Biol Toxicol 26(5):421–434. doi:10.1007/s10565-010-9153-7

Abdelhamid G, Anwar-Mohamed A, Elmazar MM, El-Kadi AO (2010b) Modulation of NAD(P)H:quinone oxidoreductase by vanadium in human hepatoma HepG2 cells. Toxicol In Vitro Int J Publ Assoc BIBRA 24(6):1554–1561. doi:10.1016/j.tiv.2010.06.017

Abdelhamid G, Amara IE, Anwar-Mohamed A, El-Kadi AO (2012) Modulation of aryl hydrocarbon receptor-regulated genes by acute administration of ammonium metavanadate in kidney, lung and heart of C57BL/6 mice. J App Toxicol JAT doi:10.1002/jat.2774

Amara IE, Anwar-Mohamed A, Abdelhamid G, El-Kadi AO (2012) Mercury modulates the cytochrome P450 1a1, 1a2 and 1b1 in C57BL/6 mice: in vivo and in vitro studies. Toxicol Appl Pharmacol 266(3):419–429. doi:10.1016/j.taap.2012.11.027

Amoyal I, Prus E, Fibach E (2007) Vanadate elevates fetal hemoglobin in human erythroid precursors by inhibiting cell maturation. Exp Biol Med (Maywood) 232(5):654–661

Anwar-Mohamed A, El-Kadi AO (2008) Down-regulation of the carcinogen-metabolizing enzyme cytochrome P450 1a1 by vanadium. Drug Metab Dispos Biol Fate Chem 36(9):1819–1827. doi:10.1124/dmd.108.021154

Anwar-Mohamed A, El-Kadi AO (2009) Down-regulation of the detoxifying enzyme NAD(P)H:quinone oxidoreductase 1 by vanadium in Hepa 1c1c7 cells. Toxicol Appl Pharmacol 236(3):261–269

Anwar-Mohamed A, El-Kadi AO (2010) Arsenite down-regulates cytochrome P450 1A1 at the transcriptional and posttranslational levels in human HepG2 cells. Free Radical Biol Med 48(10):1399–1409. doi:10.1016/j.freeradbiomed.2010.02.027

Barakat MM, El-Kadi AO, du Souich P (2001) L-NAME prevents in vivo the inactivation but not the down-regulation of hepatic cytochrome P450 caused by an acute inflammatory reaction. Life Sci 69(13):1559–1571

Baran EJ (2008) Vanadium detoxification: chemical and biochemical aspects. Chem Biodivers 5(8):1475–1484

Barceloux DG (1999) Vanadium. J Toxicol Clin Toxicol 37(2):265–278
Beyhl FE, Mayer DG (1983) Effects of ammonium metavanadate on rat liver enzymes. Toxicol Lett 19(1–2):81–85
Brauze D, Widerak M, Cwykiel J, Szyfter K, Baer-Dubowska W (2006) The effect of aryl hydrocarbon receptor ligands on the expression of AhR, AhRR, ARNT, Hif1alpha, CYP1A1 and NQO1 genes in rat liver. Toxicol Lett 167(3):212–220
Byrne AR, Kosta L (1978) Vanadium in foods and in human body fluids and tissues. Sci Total Environ 10(1):17–30
Dafnis E, Spohn M, Lonis B, Kurtzman NA, Sabatini S (1992) Vanadate causes hypokalemic distal renal tubular acidosis. Am J Physiol 262(3 Pt 2):F449–F453
El-Kadi AO, Maurice H, Ong H, du Souich P (1997) Down-regulation of the hepatic cytochrome P450 by an acute inflammatory reaction: implication of mediators in human and animal serum and in the liver. Br J Pharmacol 121(6):1164–1170
Evangelou AM (2002) Vanadium in cancer treatment. Crit Rev Oncol Hematol 42(3):249–265
Galloway JT, Norton J, Volchok S, MacLean H (1982) Trace metals in atmospheric deposition: a review and assessment. Atmos Environ 16:1677–1700
Germolec DR, Henry EC, Maronpot R et al (1996) Induction of CYP1A1 and ALDH-3 in lymphoid tissues from Fisher 344 rats exposed to 2,3,7,8-tetrachlorodibenzodioxin (TCDD). Toxicol Appl Pharmacol 137(1):57–66
Guengerich FP, Shimada T (1998) Activation of procarcinogens by human cytochrome P450 enzymes. Mutat Res 400(1–2):201–213
Hankinson O (1995) The aryl hydrocarbon receptor complex. Annu Rev Pharmacol Toxicol 35:307–340
Hansen TV, Aaseth J, Skaug V (1986) Hemolytic activity of vanadyl sulphate and sodium vanadate. Acta Pharmacologica et Toxicologica 59(Suppl 7):562–565
Hartree EF (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal Biochem 48(2):422–427
Hogan GR (1990) Peripheral erythrocyte levels, hemolysis and three vanadium compounds. Experientia 46(5):444–446
Klimecki WT, Carter DE (1995) Arsine toxicity: chemical and mechanistic implications. J Toxicol Environ Health 46(4):399–409. doi:10.1080/15287399509532045
Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4):402–408
Lorenzen A, Kennedy SW (1993) A fluorescence-based protein assay for use with a microplate reader. Anal Biochem 214(1):346–348
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275
Mamane Y, Pirrone N (1998) Vanadium in the atmosphere, vol Part 1: chemistry and biochemistry. Wiley, New York, NY
Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1–2):55–63
Nebert DW, Eisen HJ, Hankinson O (1984) The Ah receptor: binding specificity only for foreign chemicals? Biochem Pharmacol 33(6):917–924
Obianime AW, Gogo-Abite M, Roberts II (2009) The effects of ammonium metavanadate on biochemical, hormonal, haematological and histopathological parameters of the female Wistar rats. Niger J Physiol Sci Off Publ Physiol Soc Niger 24(2):187–194
Pohl RJ, Fouts JR (1980) A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. Anal Biochem 107(1):150–155
Roman RJ, Bonventre JV, Silva P, Lechene C (1981) Sodium orthovanadate diuresis in rats. J Pharmacol Exp Ther 218(1):168–174
Sambrook J, Fritsch EF, Maniatatis T (1989) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Plainview, NY
Seglen PO (1976) Preparation of isolated rat liver cells. Methods Cell Biol 13:29–83
Setyawati IA, Thompson KH, Yuen VG et al (1998) Kinetic analysis and comparison of uptake, distribution, and excretion of 48 V-labeled compounds in rats. J Appl Physiol 84(2):569–575
Spink DC, Katz BH, Hussain MM et al (2002) Induction of CYP1A1 and CYP1B1 in T-47D human breast cancer cells by benzo[a]-pyrene is diminished by arsenite. Drug Metab Dispos 30(3):262–269
Talvitie NA, Wagner WD (1954) Studies in vanadium toxicology. II. Distribution and excretion of vanadium in animals. AMA Arch Ind Hyg Occup Med 9(5):414–422
Uno S, Dragin N, Miller ML, Dalton TP, Gonzalez FJ, Nebert DW (2008) Basal and inducible CYP1 mRNA quantitation and protein localization throughout the mouse gastrointestinal tract. Free Radic Biol Med 44(4):570–583
Vakharia DD, Liu N, Pause R et al (2001) Effect of metals on polycyclic aromatic hydrocarbon induction of CYP1A1 and CYP1A2 in human hepatocyte cultures. Toxicol Appl Pharmacol 170(2):93–103. doi:10.1006/taap.2000.9087
Vazquez A, Tudela J, Varon R, Garcia-Canovas F (1991) Determination of hemoglobin through its peroxidase activity on chlorpromazine. J Biochem Biophys Methods 23(1):45–52
Volotinen M, Maenpaa J, Kankuri E et al (2009) Expression of cytochrome P450 (CYP) enzymes in human nonpigmented ciliary epithelial cells: induction of CYP1B1 expression by TCDD. Invest Ophthalmol Vis Sci 50(7):3099–3105
Yang CS, Smith TJ, Hong JY (1994) Cytochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: opportunities and limitations. Cancer Res 54(7 Suppl): 1982s–1986s