http://informahealthcare.com/xen
ISSN: 0049-8254 (print), 1366-5928 (electronic)

Xenobiotica

Xenobiotica, Early Online: 1–11
© 2013 Informa UK Ltd. DOI: 10.3109/00498254.2012.754113

informa healthcare

RESEARCH ARTICLE

Acute arsenic treatment alters cytochrome P450 expression and arachidonic acid metabolism in lung, liver and kidney of C57Bl/6 mice

Anwar Anwar-Mohamed, Ahmed El-Sherbeni, Seok Hee Kim, Osama H. Elshenawy, Hassan N. Althurwi, Beshay N. M. Zordoky, and Ayman O. S. El-Kadi

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

Abstract

1. Arsenic (As(III)) toxicity has received increasing attention as human exposure to arsenic is associated with pulmonary, hepatic and renal toxicities. Therefore, in the present study, we investigated the effect of acute As(III) treatment on pulmonary, hepatic and renal cytochrome (CYP) P450-mediated arachidonic acid metabolism.
2. Our results demonstrated that acute As(III) treatment (12.5 mg/kg) altered CYP epoxygenases, CYP ω-hydroxylases and EPHX2 mRNA levels that were isozyme and tissue specific.
3. Furthermore, As(III) increased the formation of epoxyeicosatrienoic acids (EETs) in the kidney without affecting their levels in the lung or liver. In addition, acute As(III) treatment increased dihydroxyeicosatrienoic acid (DHETs) formation in the lung, while it did not affect liver DHETs formation and decreased kidney DHETs formation.
4. As(III) also increased total epoxygenases activity in the lung while it decreased its levels in the kidney and had no effect on the liver. Furthermore, As(III) increased 20-hydroxyeicosatetraenoic acid formation in the liver while it decreased its formation in the kidney.
5. Lastly, As(III) increased soluble epoxide hydrolase activity in the lung, while it decreased its levels in the kidney and had no effect on the liver. In conclusion, this is the first demonstration that As(III) alters arachidonic acid metabolism in a tissue specific manner.

Keywords

Arsenite, cytochrome epoxygenases, cytochrome ω-hydroxylases, epoxyeicosatrienoic acid, soluble epoxide hydrolase, 20-hydroxyeicosatetraenoic acid

History

Received 2 October 2012
Revised 23 November 2012
Accepted 26 November 2012
Published online 15 February 2013

Introduction

Arsenite (As(III)) toxicity has received increasing attention as human exposure to arsenic was associated with pulmonary, hepatic and renal toxicities (Kumazaki et al., 2011; Mathews et al., 2006; Patel & Kalia, 2010; Rahman et al., 2009). In general, As(III)-induced toxic effects are mainly attributed to the induction of oxidative stress and apoptosis (Zhao et al., 2008). As such, exposure to As(III) has also been associated with multiple respiratory problems including bronchitis, shortness of breath and cough (Rahman et al., 2009). Similarly, in the liver As(III) exposure causes hepatotoxic effects including hepatomegaly, portal fibrosis and cirrhosis (Mazumder, 2005). Renal dysfunctions have been also reported in individuals exposed to As(III) as target sites for As(III)-mediated toxic effects in the kidney include capillaries, tubules and glomeruli (Patel & Kalia, 2010). Despite these numerous toxic effects of As(III), the mechanisms behind these toxic effects are yet to be determined.

We have previously shown that As(III)-induced cardiotoxicity induces soluble epoxide hydrolase (sEH) and several cytochrome P450 (CYP) enzymes in the heart of male C57Bl/6 mice as well as in the heart-derived H9c2 cells with subsequent alteration of cytochrome CYP enzymes (CYP)-mediated arachidonic acid metabolism (Anwar-Mohamed et al., 2012). Previous reports have shown that As(III) is capable of altering the expression of several CYPs in the lung, liver and the kidney (Davey et al., 2008; Manimaran et al., 2010; Ramanathan et al., 2003; Wu et al., 2009). However, none of these studies investigated the subsequent effects of As(III) on CYP-mediated arachidonic acid metabolism in these organs. CYP enzymes play an important role in arachidonic acid metabolism in addition to the cycloxygenase and the lipoxygenase pathways (Roman, 2002). Although the role of CYP-derived arachidonic acid metabolites in the cardiovascular physiology and pathophysiology grabbed the major scientific attention (Elbekai & El-Kadi, 2006), their roles in the lung, liver and kidney cannot be ignored.

CYP enzymes are abundantly expressed in the lung, liver and the kidney where they are involved in the metabolism of exogenous as well as endogenous compounds (Danielson, 2002). With regard to arachidonic acid metabolism, pulmonary, hepatic and renal CYP epoxygenases metabolize arachidonic acid to different epoxyeicosatrienoic

Address for correspondence: Ayman O. S. El-Kadi, PhD, Faculty of Pharmacy and Pharmaceutical Sciences, 2142J Katz Group-Rexall Centre for Pharmacy and Health Research, University of Alberta, Edmonton, Alberta, T6G 2E1, Canada. Tel: 780-492-3071. Fax: 780-492-1217. E-mail: aelkadi@ualberta.ca

acid (EET) regioisomers, while CYP hydroxylases metabolize it to hydroxyeicosatetraenoic acids (HETEs) (Imaoka et al., 2005; Kaspera & Totah, 2009; Roman, 2002; Sacerdoti et al., 2003; Schwartzman et al., 1990). Furthermore, sEH enzyme which catalyzes the conversion of EETs to the less biologically active dihydroxyeicosatrienoic acids (DHETs) is also abundantly expressed in the heart, lung, liver and kidney in both human and experimental animals (Enayetallah et al., 2004; Zordoky et al., 2008).

Therefore, many investigators have addressed the role of CYP-derived arachidonic acid metabolites in the pulmonary, hepatic and the renal functions (Maier & Roman, 2001; Sacerdoti et al., 2003). Experimental evidence indicates that all of the EET regioisomers elicit pulmonary vasoconstriction, while endothelium-derived 20-HETE elicits vasodilatation (Birks et al., 1997; Loot & Fleming, 2011). Nevertheless, little information is known about the role of these eicosanoids in the liver (Sacerdoti et al., 2003). As such, 11,12-EET has been shown to have a vasoconstrictive effect on the porto-sinusoidal circulation in rat, while 20-HETE showed a weaker vasoconstricting effect which was cycloxygenase dependent (Sacerdoti et al., 2003). Moreover, EETs were also shown to be involved in vasopressin-induced glycogenolysis in rat hepatocytes (Yoshida et al., 1990). In the kidney, however, both EETs and 20-HETE have a diuretic effect through inhibiting sodium reabsorption in the proximal tubule (Moreno et al., 2001).

Many investigators have reported the modulation of pulmonary, hepatic and renal CYP-mediated arachidonic acid metabolism in several pathophysiological and experimental conditions (Anwar-Mohamed et al., 2010; Qu et al., 1998; Theken et al., 2011; Yu et al., 2006). However, there is no information about the effect of As(III) treatment on the expression of CYP enzymes in the lung, liver and the kidney. Therefore, in the present study, we investigated the effect of acute As(III) treatment on the expression of several CYP and sEH enzymes in the lung, liver and kidney of male C57Bl/6 mice. In addition, we investigated the effect of acute As(III) treatment on the formation of arachidonic acid metabolites to determine whether the changes in CYP and sEH expression have led to changes in CYP-mediated arachidonic acid metabolites.

Materials and methods

Materials

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. (San Diego, CA) according to previously published sequences. Arachidonic acid, 4-hydroxybenzophenone and sodium arsenite were purchased from Sigma-Aldrich (St. Louis, MO). Arachidonic acid metabolites standards 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, 5,6-DHET, 8,9-DHET, 11,12-DHET, 14,15-DHET, 19-HETE and 20-HETE were obtained from Cayman Chemical (Ann Arbor, MI). Reagents used for liquid chromatographic-electron spray ionization-mass spectrometry (LC-ESI-MS) were at HPLC-grade. Acetonitrile and water (HPLC grade) were purchased from EM Scientific (Gibbstawn, NJ). Acrylamide, *N′N′*-bis-methylene-acrylamide, ammonium persulfate, β-mercaptoethanol, glycine, nitrocellulose membrane (0.45 µm) and TEMED were purchased from Bio-Rad Laboratories (Hercules, CA). Chemiluminescence Western blotting detection reagents were purchased from GE Healthcare Life Sciences (Piscataway, NJ). Other chemicals were purchased from Fisher Scientific Co. (Toronto, ON, Canada).

Animals

All experimental procedures involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. Male C57Bl6 mice weighing 25–30 g were obtained from Charles River Canada (St. Constant, QC, Canada). Animals were treated intraperitoneally (IP) with a single 12.5 mg/kg As(III) ($n = 12$). Weight-matched controls received the same volume of normal saline ($n = 12$). Animals were euthanized 6 and 24 h following the injection under isoflurane anesthesia. All animals were allowed free access to food and water throughout the treatment period. The lungs, livers and kidneys were excised, immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

RNA extraction and cDNA synthesis

Total RNA from the frozen tissues was isolated using TRIzol reagent (Invitrogen, Burlington, ON, Canada) 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 10X RT buffer, 0.8 µl 25X dNTP mix (100 mM), 2.0 µl 10X RT random primers, 1.0 µl MultiScribe™ reverse transcriptase, and 3.2 µl nuclease-free water. The final reaction mix was kept at 25 °C for 10 min, heated to 37 °C for 120 min, heated for 85 °C for 5 s, and finally cooled to 4 °C.

Quantification by real-time PCR

Quantitative analysis of specific mRNA expression was performed by real-time PCR, by subjecting the resulting cDNA to PCR amplification using 96-well optical reaction plates in the ABI Prism 7500 System (Applied Biosystems). 25-µl reaction mix contained 0.1 µl of 10 µM forward primer and 0.1 µl of 10 µM reverse 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 were chosen from previously published studies and are listed in Table 1. No-template controls were incorporated onto the same plate to test for the contamination of any assay reagents. An optical adhesive cover was used to seal the plate; thereafter, 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. Dissociation curves were performed by the end of each cycle to confirm the specificity of the primers and the purity of the final PCR product.

Table 1. Primers sequences used for real-time PCR reactions.

Gene	Forward primer	Reverse primer
B-actin	5′-TAT TGG CAA CGA GCG GTT CC-3′	5′-GGC ATA GAG GTC TTT ACG GAT GTC-3′
Cyp1b1	5′-AAT GAG GAG TTC GGG CGC ACA-3′	5′-GGC GTG TGG AAT GGT GAC AGG-3′
Cyp2b10	5′-GGG AAC CTC TTG CAG ATG-3′	5′-CCC AGG TGC ACT GTG AA-3′
Cyp2b19	5′-CAC AAA GCC TTC CTC ACC GAT-3′	5′-ACA AGC AAG CAA CCC ACA CTC-3′
Cyp2b9	5′-GCT GCA GCT CAG CTA GTT ATG C-3′	5′-GCC CAC TGG CAA AAA ATA TAC C-3′
Cyp2c29	5′-TGG TCC ACC CAA AAG AAA TTG A-3′	5′-GCA GAG AGG CAA ATC CAT TCA-3′
Cyp2c38	5′-GCA TTA CTT TTA GCA ATG GAA ACA GT-3′	5′-CCA CAA GAC ACT GTG CTT CTT CTC-3′
Cyp2c40	5′-TCC GGT TTT TGA CAA GGT TTC TAC-3′	5′-TGC CCA AGT TCC TCA AGG TAT TC-3′
Cyp2c44	5′-CTT TTC AAC GAG CGA TTC CC-3′	5′-TGT TTC TCC TCC TCG ATC TTG C-3′
Cyp2e1	5′-CCC AAG TCT TTA ACC AAG TTG GC-3′	5′-CTT CCA TGT GGG TCC ATT ATT GA-3′
Cyp2j11	5′-GTA TGA TGG ACA GTC ACC GGG A-3′	5′-GGT CCA GAG CAG TGC AGA TGA-3′
Cyp2j13	5′-GGG AAG AGG AAG GAC AGC CTT-3′	5′-GCA GCA GCT CCT GAA ACT GAC T-3′
Cyp2j5	5′-TGT GAA TCG CTT TAT GAC ACC G-3′	5′-TGA TGG GTC TCC TCC TGA ATG-3′
Cyp2j9	5′-GGG AAT GTT CTA AGC CTG GAT TT-3′	5′-GAG TGA CTG GGC GAT TCA TAA A-3′
Cyp4a10	5′-GTG CTG AGG TGG ACA CAT TCA T-3′	5′-TGT GGC CAG AGC ATA GAA GAT C-3′
Cyp4a12	5′-TGA CCC CAG CTT TCC ACT ATG-3′	5′-TTG TTC AGG TCC TCA ACT GCC-3′
Cyp4a14	5′-GTC TCT CGG GGA GCA ATA TAC G-3′	5′-ACC AAT CCA GGG AGC AAA GAA-3′
Cyp4f13	5′-CCC TAA ACC GAG CTG GTT CTG-3′	5′-GAG TCG CAG GAT TGG GTA CAC-3′
Cyp4f15	5′-CCT GTG CTG TGT TCC TTA GGG-3′	5′-GAC GGG AAA TGA CCG TGA CT-3′
Cyp4f16	5′-CCG CCT CAG TTG TTT CCC TC-3′	5′-TGC CCA AGT GAC CTG AAA ACC-3′
Cyp4f18	5′-CTG CAT CCT CCC GTC ACT G-3′	5′-GGA TTG TGA TGT GTC CCG AAA-3′
EPHX2	5′-GAA AGG ATT CAC AAC ATG CAT TG-3′	5′-GGC CAG GCT GTC TCT CTT GTC-3′

Real-time PCR data analysis

The real-time PCR data were analyzed using the relative gene expression method as described in Applied Biosystems User Bulletin No. 2. Briefly, the data are presented as the fold change in gene expression normalized to the endogenous reference gene (β-actin) and relative to the untreated control of the same time point.

Preparation of microsomal and cytosolic protein fractions

Microsomal protein was prepared from the lung, liver and kidney tissues as described previously (Barakat et al., 2001). Briefly, lung, liver and kidney tissues were washed in ice-cold KCL (1.15% w/v), 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, microsomes, were reconstituted in cold sucrose, and the supernatant, cytosols, were all stored at −80 °C. Lung, liver and kidney 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 (Anwar-Mohamed et al., 2010). Briefly, 10 µg of cytosol for sEH, or 20 µg of microsomes for Cyp2c, Cyp2j and Cyp4a 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 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 rabbit anti-mouse Cyp2c, primary polyclonal rabbit anti-mouse Cyp2j, primary polyclonal mouse anti-mouse Cyp4a, primary polyclonal goat anti-mouse sEH, primary polyclonal rabbit anti-mouse actin or primary polyclonal goat anti-mouse Gapdh, all antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Overnight at 4 °C incubation with a peroxidase-conjugated goat anti-rabbit IgG secondary antibody for Cyp2c, Cyp2j and actin or goat anti-mouse IgG secondary antibody for Cyp4a, or rabbit anti-goat IgG secondary antibody for sEH 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 separation of different arachidonic acid metabolites by LC-ESI-MS

Lung, liver and kidney microsomes (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. Arachidonic acid was added to a final concentration of 50 µM and incubated for 30 min. The reaction was terminated by the addition of 600 µl ice-cold acetonitrile followed by the internal standard, 4-hydroxybenzophenone. Arachidonic acid metabolites were extracted by 1 ml ethyl acetate twice and dried using speed vacuum (Savant, Farmingdale, NY). Extracted arachidonic acid metabolites were analyzed using LC-ESI-MS (Waters Micromass ZQ 4000 spectrometer, Waters, Milford, MA) method as described previously (Aboutabl et al., 2009). Briefly, the mass spectrometer was

operated in negative ionization mode with single ion recorder acquisition. The nebulizer gas was acquired from an in-house high purity nitrogen source. The source temperature was set at 150 °C, and the voltages of the capillary and the cone were 3.51 KV and 25 V, respectively. The samples (10 µl) were separated on reverse phase C18 column (250 × 3.2 mm, AkzoNobel/Kromasil, Brewster, New York) using linear gradient mobile phase system with a mobile phase of water/acetonitrile with 0.005% acetic acid at flow rate of 0.2 ml/min. The mobile phase system started at 60% acetonitrile, linearly increased to 80% acetonitrile in 30 min, increased to 100% acetonitrile in 5 min and held for 5 min.

sEH activity assay

sEH activity was measured using the Morisseau and Hammock method with modifications. 14,15-EET was used as the natural substrate (Morisseau & Hammock, 2007). Briefly, the cytosolic fraction was diluted to 0.4 mg/ml for lung, and 0.04 mg/ml for liver and kidney with sodium phosphate buffer (0.076 M, pH 7.4) supplemented with BSA (2.5 mg/ml). The assay was initiated by the addition of 14,15-EET (final concentration of 14,15-EET is 2 µg/ml) final volume of incubates is 200 µl. The mixture was incubated at 37 °C for 10 min. The reaction was terminated by the addition of 600 µl ice-cold acetonitrile followed by the internal standard, 4-hydroxybenzophenone. 14,15-EET and its corresponding 14,15-DHET were extracted by 1 ml ethyl acetate twice and dried using speed vacuum (Savant, Farmingdale, NY). Extracted 14,15-EET and its metabolite were analyzed using LC-ESI-MS (Waters Micromass ZQ 4000 spectrometer) method as described previously (Zordoky et al., 2010).

Statistical analysis

Data are presented as mean ± standard error (SE) of the mean. Control and treatment measurements were compared using Student's t test. A result was considered statistically significant where $p < 0.05$.

Results

Effect of As(III) treatment on CYP gene and protein expression

To examine the effect of As(III) treatment on the gene expression profile of CYP epoxygenases and ω-hydroxylases in the lung, liver and the kidney, total RNA was extracted from the lung, liver and the kidney of both control and As(III)-treated mice. Thereafter, the expression of different genes was measured using reverse transcription followed by real-time PCR as described under materials and methods.

Table 2 shows that As(III) significantly induced Cyp1b1 mRNA levels in the lung, liver and the kidney by 1.60-, 2.70- and 1.50-fold, respectively compared to control (Table 2). With regard to Cyp2 family, As(III) treatment significantly induced Cyp2b9 mRNA levels in the liver and the kidney by 3.60- and 3.50-fold, respectively, while it did not significantly affect lung Cyp2b9 as compared to control (Table 2). Similarly, Cyp2b10 mRNA levels were significantly induced in the lung, liver and the kidney of As(III) treated animals by 2.30-, 2.60- and 3.20-fold, respectively, compared to control (Table 2). Furthermore, As(III) treatment significantly induced Cyp2b19 mRNA levels in the lung and the kidney by 1.60- and 2.20-fold, respectively, while it did not significantly affect liver Cyp2b19 mRNA levels as compared to control (Table 2).

Table 2. Effect of As(III) on Cyps gene expression in lung, liver and kidney.

Cyp	Treatment	Lung	Liver	Kidney
Cyp1b1	Control	1 ± 0.15	1 ± 0.09	1 ± 0.13
	As(III)	1.59 ± 0.09[a]	2.70 ± 0.51[a]	1.50 ± 0.11[a]
Cyp2b9	Control	1 ± 0.07	1 ± 0.20	1 ± 0.15
	As(III)	1.34 ± 0.24	3.56 ± 1.10[a]	3.52 ± 1.14[a]
Cyp2b10	Control	1 ± 0.08	1 ± 0.09	1 ± 0.10
	As(III)	2.26 ± 0.23[a]	2.58 ± 0.22[a]	3.15 ± 0.56[a]
Cyp2b19	Control	1 ± 0.07	1 ± 0.19	1 ± 0.07
	As(III)	1.61 ± 0.07[a]	1.23 ± 0.29	2.20 ± 0.14[a]
Cyp2c29	Control	1 ± 0.21	1 ± 0.09	1 ± 0.16
	As(III)	0.28 ± 0.07[a]	2.49 ± 0.33[a]	374.54 ± 29.19[a]
Cyp2c38	Control	1 ± 0.14	1 ± 0.10	1 ± 0.07
	As(III)	0.21 ± 0.04[a]	4.45 ± 1.03[a]	0.96 ± 0.21
Cyp2C40	Control	1 ± 0.15	1 ± 0.14	1 ± 0.07
	As(III)	0.06 ± 0.02[a]	2.24 ± 0.37[a]	1.47 ± 0.59[a]
Cyp2c44	Control	1 ± 0.14	1 ± 0.12	1 ± 0.10
	As(III)	1.06 ± 0.17	0.54 ± 0.04[a]	0.68 ± 0.12
Cyp2e1	Control	1 ± 0.18	1 ± 0.06	1 ± 0.08
	As(III)	0.15 ± 0.02[a]	0.66 ± 0.04[a]	0.17 ± 0.04[a]
Cyp2j5	Control	1 ± 0.14	1 ± 0.04	1 ± 0.04
	As(III)	0.19 ± 0.07[a]	2.01 ± 0.33[a]	0.76 ± 0.07
Cyp2j9	Control	1 ± 0.07	1 ± 0.13	1 ± 0.05
	As(III)	2.00 ± 0.19[a]	15.77 ± 1.10[a]	1.94 ± 0.15[a]
Cyp4a10	Control	1 ± 0.09	1 ± 0.11	1 ± 0.08
	As(III)	0.84 ± 0.07	1.96 ± 0.24[a]	0.80 ± 0.06
Cyp4a14	Control	1 ± 0.15	1 ± 0.12	1 ± 0.19
	As(III)	0.19 ± 0.06[a]	3.82 ± 0.49[a]	1.39 ± 0.21
Cyp4f13	Control	1 ± 0.09	1 ± 0.06	1 ± 0.04
	As(III)	1.37 ± 0.078[a]	1.51 ± 0.13[a]	0.94 ± 0.03
Cyp4f15	Control	1 ± 0.08	1 ± 0.08	1 ± 0.10
	As(III)	1.029 ± 0.12	2.97 ± 0.58[a]	1.85 ± 0.07[a]
Cyp4f16	Control	1 ± 0.03	1 ± 0.09	1 ± 0.04
	As(III)	0.94 ± 0.05	3.15 ± 0.56[a]	0.69 ± 0.07
Cyp4f18	Control	1 ± 0.10	1 ± 0.08	1 ± 0.07
	As(III)	1.97 ± 0.30[a]	1.18 ± 0.48	1.56 ± 0.20[a]

[a] $p < 0.05$ compared to control.

As(III) treatment also significantly induced liver and kidney Cyp2c29 mRNA levels by 2.50- and 374.50-fold, respectively, while it significantly inhibited lung Cyp2c29 mRNA levels by 0.73-fold as compared to control (Table 2). As(III) significantly induced liver Cyp2c38 by 4.40-fold, while it did not significantly affect kidney Cyp2c38 mRNA levels and significantly inhibited lung Cyp2c38 mRNA levels by 0.8-fold, compared to control (Table 2). Furthermore, As(III) significantly induced liver and kidney Cyp2c40 by 2.20- and 1.50-fold, respectively, while it significantly inhibited lung Cyp2c40 by 0.94-fold, compared to control (Table 2). Lastly, As(III) failed to significantly affect Cyp2c44 mRNA levels in the lung or the kidney, while it significantly inhibited its level in the liver by 0.46-fold, compared to control (Table 2). Regarding the effect of As(III) on Cyp2e1 mRNA levels, As(III) significantly inhibited lung, liver, and kidney Cyp2e1 mRNA levels by 0.85-, 0.34- and 0.83-fold, respectively, compared to control (Table 2). For the Cyp2j subfamily, As(III) significantly inhibited lung Cyp2j5 mRNA levels by 0.81-fold, while it did not significantly affect kidney Cyp2j5 mRNA levels and significantly induced liver Cyp2j5 mRNA levels by 2-fold, compared to control (Table 2).

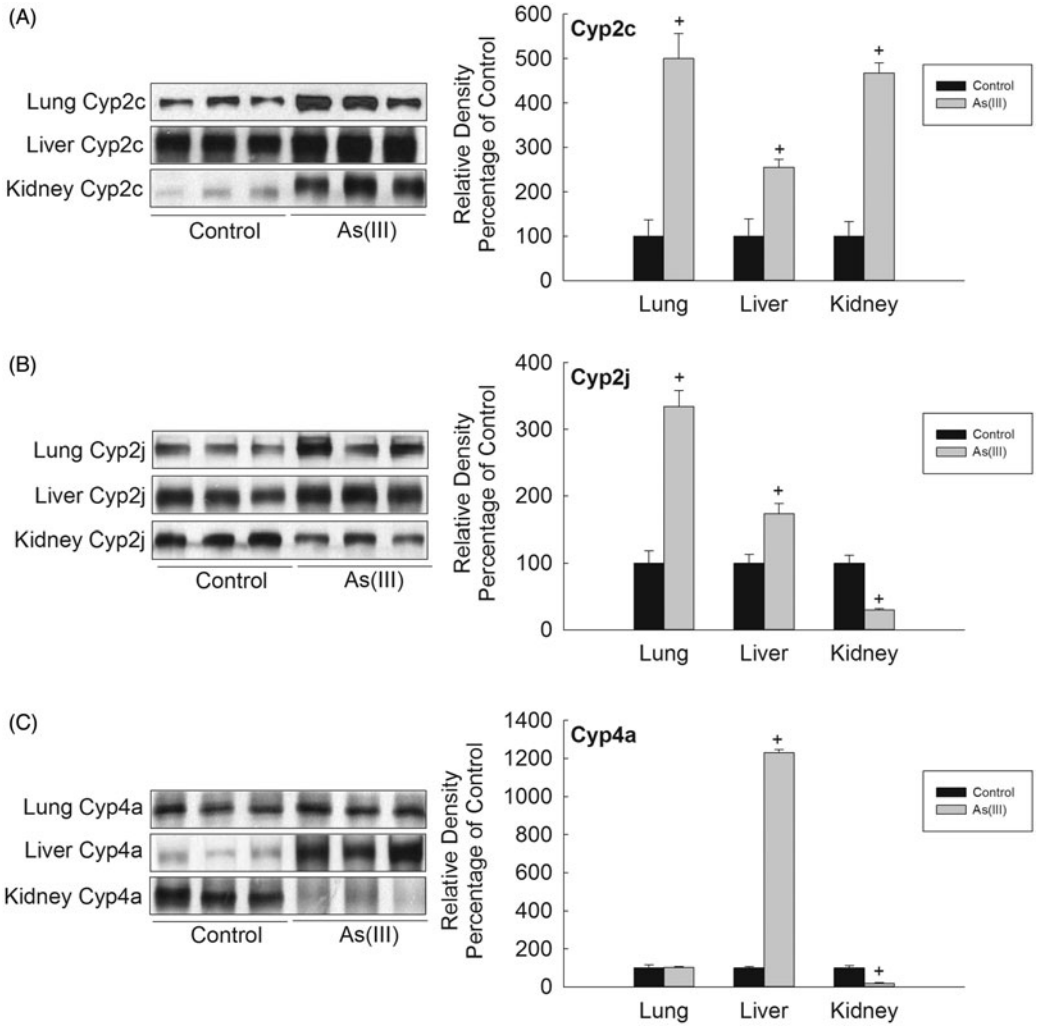

Figure 1. Effect of As(III) toxicity on protein expression of: Cyp2c (A), Cyp2j (B) and Cyp4a (C). Microsomal protein was isolated from lung, liver and kidney of control and animals treated with As(III) for 24 h. Microsomal protein (20 μg) was separated on a 10% SDS-PAGE. Cyp2c, Cyp2j, Cyp4a and actin proteins were detected by the enhanced chemiluminescence method. The graph represents the relative amount of protein normalized to the loading control (data not shown) (mean ± SE, $n = 3$), and the results are expressed as percentage of the control values taken as 100%. $^{+}p < 0.05$ compared with control.

Of interest, As(III) significantly induced lung, liver and kidney Cyp2j9 mRNA levels by 2.00-, 15.80- and 1.90-fold, respectively, compared to control (Table 2).

With regard to the major CYP ω-hydroxylases, the gene expression of *Cyp4a* and *Cyp4f* was assessed. As(III) administration significantly induced liver Cyp4a10 mRNA levels by 2-fold while it failed to significantly affect the lung or the kidney Cyp4a10 mRNA levels, compared to control (Table 2). Importantly, As(III) significantly inhibited lung Cyp4a14 mRNA levels by 0.81-fold, while it did not significantly affect its level in the kidney and significantly induced its level in the liver by 3.80-fold (Table 2). In a different pattern to that of Cyp4a, acute As(III) treatment caused a significant induction of lung and liver Cyp4f13 by 1.40- and 1.50-fold, respectively, while it did not significantly affect kidney Cyp4f13 mRNA levels (Table 2). As(III) administration caused a significant induction of liver and kidney Cyp4f15 by 3.00- and 1.85-fold, while it did not significantly affect lung Cyp4f15 mRNA levels, compared to control (Table 2). Furthermore, As(III) significantly induce liver Cyp4f16 by 3.20-fold while it did not significantly affect lung or kidney Cyp4f16 mRNA levels compared to control (Table 2). Lastly, As(III) significantly induced Cyp4f18 in the lung and the kidney by 2.00- and 1.55-fold, while it did not significantly affect liver Cyp4f18 mRNA levels, compared to control (Table 2).

To investigate whether the effect of As(III) treatment on *CYP* gene expression was further translated to the protein level, microsomal protein was prepared from lung, liver and kidney of control and mice treated with As(III) for 24 h. Thereafter, Cyp2c, Cyp2j and Cyp4a protein expression levels were determined using Western blot analysis relative to actin as an endogenous control (data not shown). Our results demonstrated that As(III) significantly induced Cyp2c protein expression levels in lung, liver and kidney by 4.00-, 1.55- and 2.70-fold, respectively, compared to control (Figure 1A). Furthermore, As(III) significantly induced Cyp2j protein expression levels in lung and liver by 2.30- and 0.70-fold, respectively, compared to control while it significantly inhibited Cyp2j protein expression levels in the kidney by 0.70-fold compared to control (Figure 1B). As(III) did not significantly affect lung Cyp4a protein expression levels, while it significantly induced liver Cyp4a protein expression levels by 11-fold and significantly inhibited kidney Cyp4a

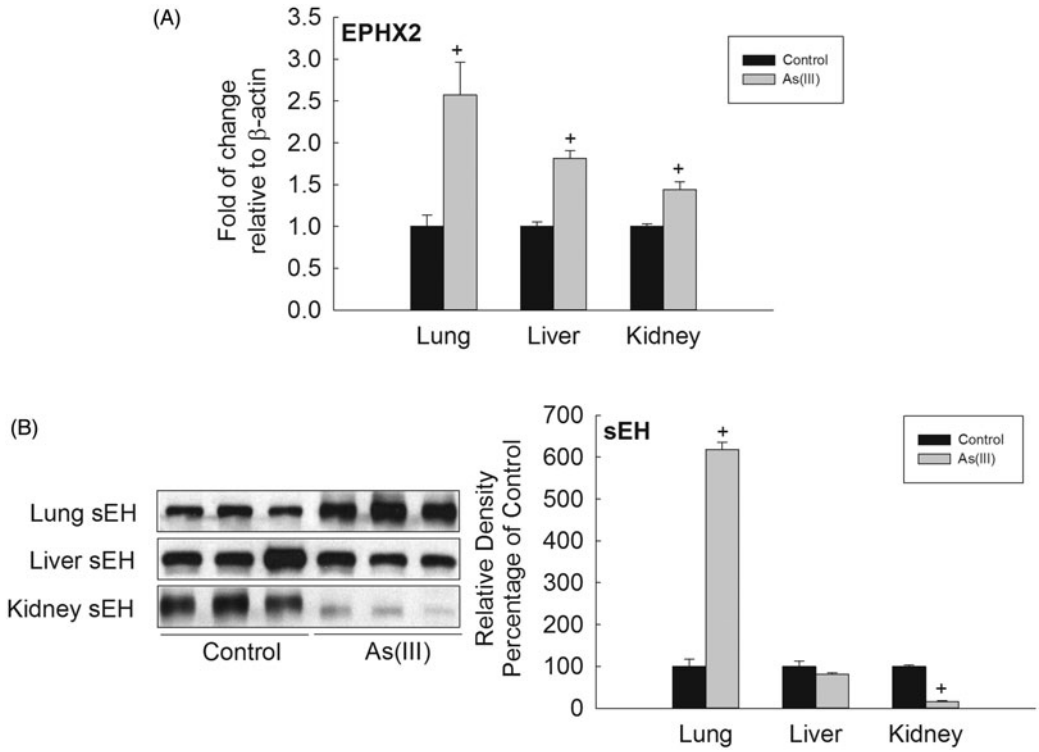

Figure 2. Effect of As(III) toxicity on: cardiac *EPHX2* gene expression (A) and sEH protein expression (B). (A) Total RNA was isolated from lung, liver and kidney of control and animals treated with As(III) for 6 h. Gene expressions were determined by real-time PCR. Results are presented as mean ± SE ($n = 6$). $^{+}p < 0.05$ compared to control. (B) Cytosolic protein from lung, liver and kidney was isolated from control and animals treated with As(III) for 24 h. Cytosolic protein (10 μg) was separated on a 10% SDS-PAGE. sEH and Gapdh proteins were detected by the enhanced chemiluminescence method. The graph represents the relative amount of protein normalized to the loading control (data not shown) (mean ± SE, $n = 3$), and the results are expressed as percentage of the control values taken as 100%. $^{+}p < 0.05$ compared with control.

protein expression levels by 0.80-fold compared to control (Figure 1C).

Effect of As(III) treatment on *EPHX2* gene and sEH protein expression

We also examined the effect of acute As(III) treatment on the expression of the *EPHX2* gene which encodes for the sEH enzyme. In the current study, acute As(III) treatment significantly induced *EPHX2* gene expression in the lung, liver and the kidney by 1.50-, 0.80- and 0.40-fold, respectively, compared to control (Figure 2A). To investigate whether the effect of As(III) treatment on *EPHX2* gene expression was further translated to the protein level, cytosolic protein was prepared from lung, liver and kidney of control and mice treated with As(III) for 24 h. Thereafter, sEH protein expression level was determined using Western blot analysis relative to Gapdh as an endogenous control (data not shown). Our results demonstrated that As(III) significantly induced lung sEH protein expression levels by 5.20-fold while it did not significantly affect liver sEH protein expression levels and significantly inhibited kidney sEH protein expression levels by 0.85-fold, compared to control (Figure 2B).

Effect of As(III) treatment on EETs and DHETs metabolites formation

To investigate the effect of As(III) treatment on the formation of CYP-derived arachidonic acid metabolites, lung, liver and kidney microsomes of either control or 24 h As(III)-treated mice were incubated with 50 μM arachidonic acid for 30 min. Thereafter, arachidonic acid metabolites were measured using LC-ESI-MS. In the lung and the liver microsomes of As(III)-treated mice, the formation of all EET regioisomers were non-significant than the control values (Figure 3A and B). In kidney microsomes of As(III)-treated mice, the formation of 14,15-, 11,12- and 8,9-EET were significantly higher than the control values by 5.00-, 5.20- and 1.70-fold, respectively, while the formation of 5,6-EET was significantly lower than the control value by 0.23-fold (Figure 3C).

We also measured the levels of enzymatic hydration products of EETs, the DHETs. As shown in Figure 4(A), in lung microsomes of As(III)-treated mice, the formation of 14,15-, 11,12-, 8,9- and 5,6-DHETs were significantly higher than control values by 5.40-, 0.95-, 1.85- and 3.20-fold, respectively. In liver microsomes of As(III)-treated mice, there was no significant change in the rate of DHETs formation compared to the control (Figure 4B). In the kidney microsomes of As(III)-treated mice, the formation of 14,15-, 11,12- and 8,9-DHETs were significantly lower than control values by 0.47-, 0.48- and 0.75-fold, respectively (Figure 4C).

Effect of As(III) treatment on total epoxygenases activities, ω-hydroxylases activities and sEH activities

In order to investigate the effect of As(III) on the total epoxygenase activity, we calculated the sum of all the products of epoxygenase enzymes, the total EETs and

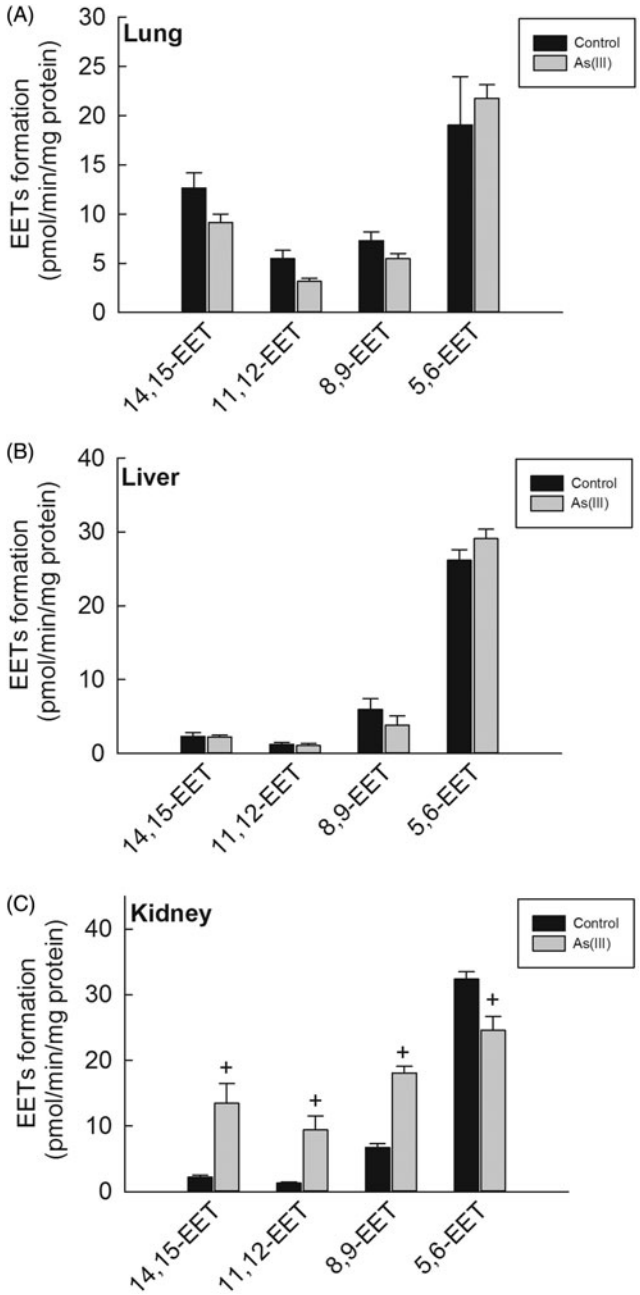

Figure 3. Effect of As(III) treatment on EETs formation: (A) lung, (B) liver and (C) kidney. Lung, liver and kidney microsomes of control or animals treated with As(III) for 24 h were incubated with 50 μM arachidonic acid. The reaction was started by the addition of 1 mM NADPH and lasted for 30 min. The reaction was terminated by the addition of ice-cold acetonitrile. EETs were extracted twice by 1 ml of ethyl acetate and dried using speed vacuum. Reconstituted metabolites were injected into LC-ESI-MS for metabolite determination. Results are presented as mean ± SE ($n = 6$). $^{+}p < 0.05$ compared with control.

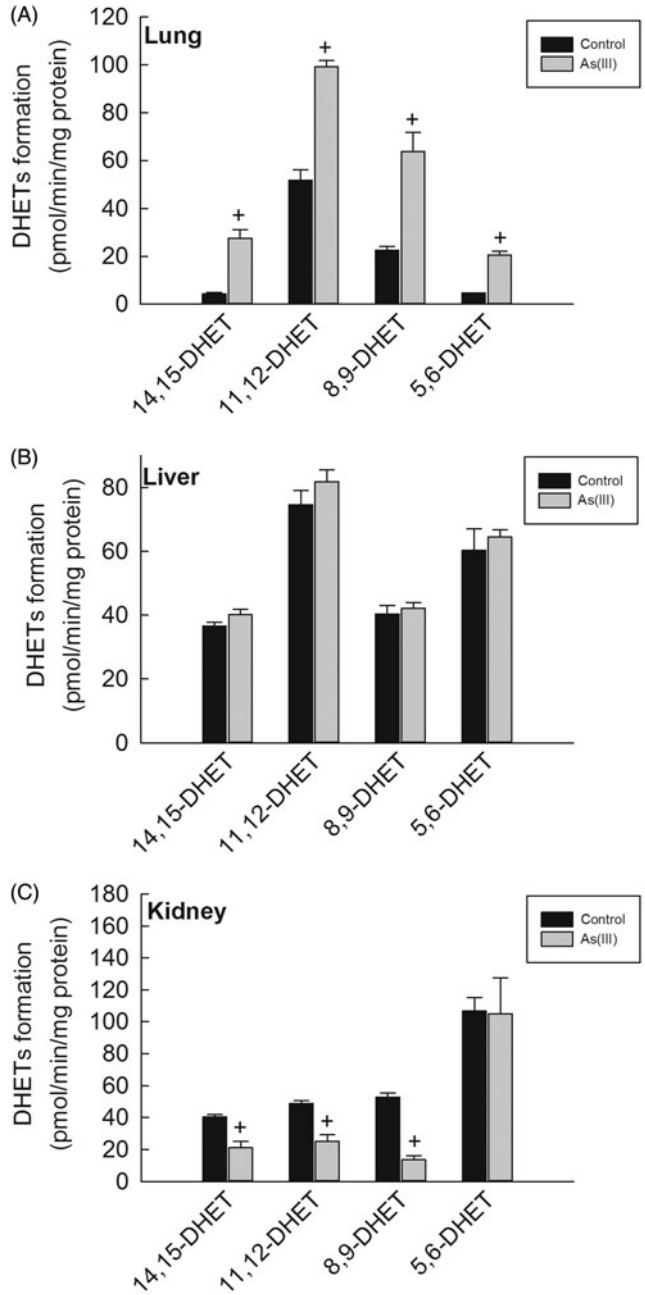

Figure 4. Effect of As(III) treatment on DHETs formation: (A) lung, (B) liver and (c) kidney. Lung, liver and kidney microsomes of control or animals treated with As(III) for 24 h were incubated with 50 μM arachidonic acid. The reaction was started by the addition of 1 mM NADPH and lasted for 30 min. The reaction was terminated by the addition of ice-cold acetonitrile. DHETs were extracted twice by 1 ml of ethyl acetate and dried using speed vacuum. Reconstituted metabolites were injected into LC-ESI-MS for metabolite determination. Results are presented as mean ± SE ($n = 6$). $^{+}p < 0.05$ compared with control.

DHETs, in control and mice treated with As(III) for 24 h. The total epoxygenase activity was not significantly altered in the liver as expected (Figure 5A). To the contrast, As(III) significantly increased total epoxygenase activity in the lung by 1.00-fold while it significantly decreased it in the kidney of As(III)-treated mice by 0.20-fold (Figure 5A). To determine the effect of As(III) treatment on ω-hydroxylases activities, we determined the rates of 20-HETE formation in the lung, liver and the kidney microsomes from control and As(III)-treated mice. As(III) treatment did not significantly affect lung 20-HETE formation (Figure 5B). On the other hand, As(III) significantly increased the liver 20-HETE formation by 4.20-fold while it completely blocked the kidney 20-HETE formation in comparison to the control group (Figure 5B). In addition, sEH activity has been assessed by determining the rate of 14,15-DHET formation from its corresponding 14,15-EET in the lung, liver and the kidney cytosols of control and mice treated with As(III) for 24 h. sEH activity significantly increased in the lung cytosols of As(III)-treated mice as compared to the control by 1.50-fold (Figure 5C).

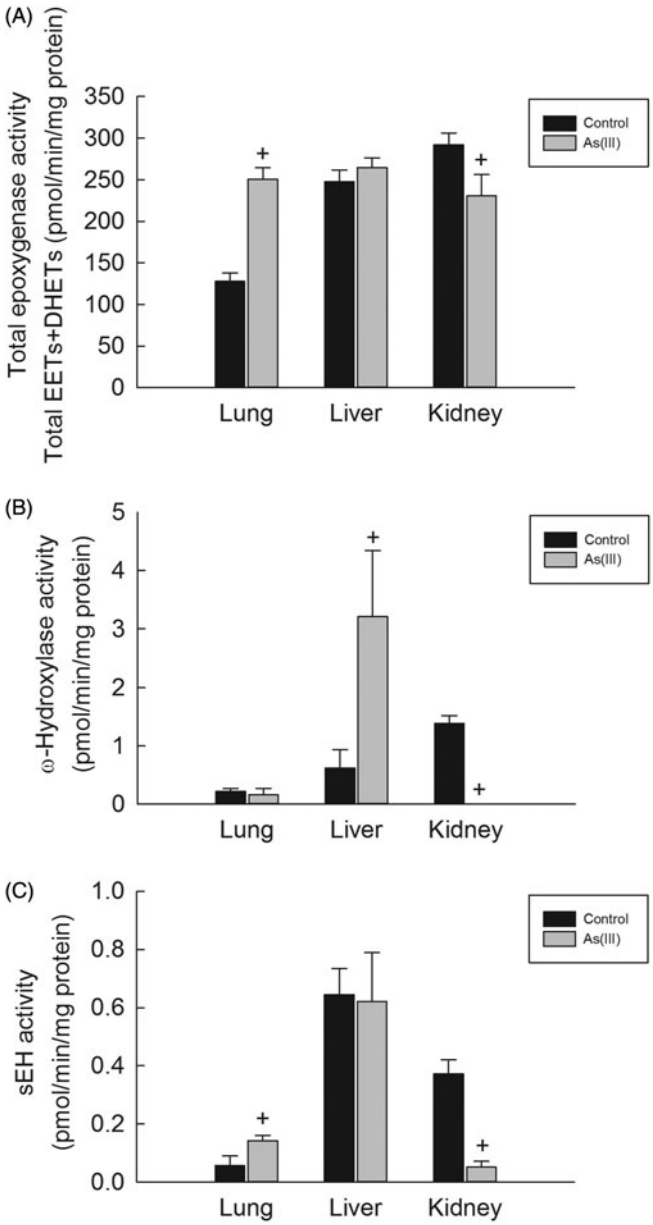

Figure 5. Effect of As(III) treatment on: (A) total epoxygenase activity, (B) 20-HETE formation and (C) sEH activity. (A and B) Lung, liver and kidney microsomes of control or animals treated with As(III) for 24 h were incubated with 50 μM arachidonic acid. The reaction was started by the addition of 1 mM NADPH and lasted for 30 min. The reaction was terminated by the addition of ice-cold acetonitrile. (C) Cytosols of lung, liver and kidney from control or animals treated with As(III) for 24 h were incubated with 50 μM 14,15-EET. EETs, DHETs and 20-HETE metabolites were extracted twice by 1 ml of ethyl acetate and dried using speed vacuum. Reconstituted metabolites were injected into LC-ESI-MS for metabolite determination. Results are presented as mean ± SE ($n = 6$). $^{+}p < 0.05$ compared with control.

Interestingly sEH activity was not changed in the liver cytosols while it significantly decreased in the kidney cytosols of As(III)-treated mice as compared to the control by 0.85-fold (Figure 5C).

Discussion

One of the physiological roles of CYP enzymes is the metabolism of arachidonic acid into EETs by CYP epoxygenases and HETEs by CYP hydroxylases (Roman, 2002). In the present study, acute As(III) treatment has been introduced by a single IP injection of 12.5 mg/kg of sodium arsenite in C57Bl/6 mice. This dose has been shown previously to induce cardiotoxicity, nephrotoxicity, and hepatotoxicity (Kimura et al., 2006; Liu et al., 2002; Yanez et al., 1991).

In the current study Cyp1b1 mRNA level was induced in all tested tissues. In this regard, it has been previously reported that constitutive *Cyp1b1* gene expression in steroidal tissues is regulated through a hormonal pathway (Bhattacharyya et al., 1995). In contrast, *Cyp1b1* gene expression in response to aryl hydrocarbon receptor (AhR)-ligands is governed by the AhR-dependent pathway (Bhattacharyya et al., 1995). In the current study, the induction of Cyp1b1 by As(III) cannot be simply attributed to the AhR-dependent pathway because As(III) is not known to be an AhR ligand. However, As(III)-induced inflammation may be a more relevant explanation to the Cyp1b1 induction as it has been previously reported that As(III) increases serum tumor necrosis factor α (TNF-α) (Das et al., 2009). Importantly, TNF-α is a potent stimulator of *Cyp1b1* gene expression in some liver cells (Piscaglia et al., 1999; Umannova et al., 2007). Mechanistically, it was postulated that inflammation induces *Cyp1b1* gene expression through the hormonal pathway (Malaplate-Armand et al., 2003). Regarding its role in arachidonic acid metabolism, Cyp1b1 can metabolize arachidonic acid to both mid-chain HETEs and EETs (Choudhary et al., 2004).

With regard to Cyp2 family, there were differential effects of As(III) on its mRNA levels across tissues. In general, acute As(III) treatment induced CYP epoxygenases of the Cyp2b, Cyp2c and Cyp2j subfamilies. The transcriptional regulation of Cyp2b and Cyp2c subfamilies has been shown to be through the constitutive androstane receptor (CAR) (Kohle & Bock, 2009). Interestingly, it has been previously demonstrated that As(III) as sodium arsenite or monosodium acid methane arsenate is capable of activating mouse CAR (Baldwin & Roling, 2009). Thus, it is plausible that the induction of Cyp2b and Cyp2c subfamilies in the current study might have occurred through the activation of CAR by As(III). On the other hand, and different from the other two Cyp2 subfamilies, Cyp2j subfamily regulation has been shown to occur through the activation of c-jun which in turn will bind to atypical activator protein-1 (AP-1) consensus in Cyp2j promoter (Marden et al., 2003). With the fact that As(III) is a potent activator of AP-1 (Felix et al., 2005), As(III) might have induced Cyp2j through activating AP-1.

In order to correlate the differential effects on CYP epoxygenases mRNA, we measured total epoxygenase activities, calculated as total EETs and DHETs in lung, liver and kidney of control and As(III) treated mice. Our results with regard to the total epoxygenase activities demonstrated that As(III) increased these activities in the lung while it did not affect its level in the liver and decreased its activity in the kidney. Although acute As(III) treatment differentially affected total epoxygenase activity in lung, liver and kidney, it was necessary to examine the effect of acute As(III) treatment on EETs and DHETs formation in these organs. In the current study, we have demonstrated for the first time that

As(III) did not affect the lung or liver EETs formation and significantly increased kidney 14,15-, 11,12- and 8,9-EETs formation. The importance of EETs as lipid mediators in the kidney has been recently reviewed (Hao & Breyer, 2007). In general, EETs with the exception of 5,6-EET enhance renal blood flow and promote salt excretion in proximal tubules through vasodilatation, and they also have a potent effect on renal vascular tone (Hao & Breyer, 2007). In contrast, it has been shown that 5,6-EET constricts the interlobular and afferent arterioles in a cycloxygenase-dependent mechanism (Imig et al., 1996). Therefore, the increase in 14,15-, 11,12- and 8,9-EETs and the decrease in 5,6-EETs might cause a vasodilative effect on the kidney. Thus, vasodilation in the kidney via altering EETs formation might be a compensatory mechanism to counteract the hypertensive effect on the heart through decreasing cardiac EETs formation.

Looking at the DHETs formation in lung, liver and kidney, acute As(III) treatment increased DHETs formation in the lung, while it did not affect liver DHETs formation and decreased kidney DHETs formation. Importantly, the formation of DHETs from their corresponding EETs is limited by two factors, the generation of EETs through CYP epoxygenases and the subsequent degradation of these EETs by sEH to their corresponding less biologically active DHETs. To examine the effect of acute As(III) treatment on sEH we sought to determine its effect on *EPHX2*, the gene encoding sEH. Our results demonstrated that EPHX2 mRNA levels were increased in all examined tissues in response to As(III) treatment. On the other hand, this effect did not match the sEH protein expression in which As(III) increased sEH protein expression in the lung, while it did not affect its levels in the liver and inhibited its level in the kidney.

In line with the protein expression results, we demonstrated that As(III) increased lung sEH activity, while it did not affect liver and decreased kidney sEH activity. Interestingly, in the lung although EETs formation levels were not changed, this does not necessarily mean that total epoxygenases and sEH activities were not changed. As such, the increase in total epoxygenases activity in the lung was accompanied by an increase in sEH activity which caused the no-effect observation on the EETs formation levels while it accumulated more DHETs as seen in Figure 4(A). In the liver there was no change in EETs formation, DHETs formation, total epoxygenases activity or sEH activity. In the kidney, however, the increase in EETs formation could be attributed to two factors, the decrease in kidney total epoxygenase activity and also the decrease in sEH activity. However, due to the relatively more pronounced decrease in sEH activity, EETs formation increased and DHETs formation decreased.

Previous results have shown that CYP ω-1-hydroxylases are mainly Cyp2e1, Cyp2j9 and Cyp4a10 (Roman, 2002). Interestingly, Cyp4a10 is also considered an ω-hydroxylase because it has the capacity to produce 20-HETE in addition to 19-HETE (Roman, 2002). As(III) inhibited Cyp2e1 mRNA levels in all tested tissues. On the other hand As(III) induced lung, liver and kidney Cyp2j9 and most of the Cyp4a and Cyp4f mRNA levels. In this context, we and other investigators have demonstrated that Cyp4a and Cyp4f enzymes are induced in the heart, kidney and liver in several models of inflammation (Anwar-Mohamed et al., 2010; Mitchell et al., 2001; Theken et al., 2011). In this regard, we have previously demonstrated that As(III) increases cyclooxygenase-2 (COX-2) indicating the presence of inflammation (Anwar-Mohamed et al., 2012). Therefore, we can attribute Cyp4a and Cyp4f induction to be mediated, at least in part, by As(III)-induced inflammation.

With regard to ω-hydroxylases activities, our results demonstrated that in the lung, 20-HETE formation was not changed which is in line with Cyp4a protein expression results. On the other hand, 20-HETE formation was increased in the liver and decreased in the kidney which is in agreement with Cyp4a protein expression results. The physiological significance of 20-HETE formation in the liver is unclear; however, the experimentally revealed vascular effects of 20-HETE suggest a potential role in regulating hepatic hemodynamics (Powell et al., 1998). Furthermore, 20-HETE has been shown to mediate cytotoxicity and apoptosis in ischemic kidney epithelial cells and its inhibition protects the kidney from ischemia/reperfusion injury (Hoff et al., 2011; Nilakantan et al., 2008). Therefore, we can conclude that the decrease in 20-HETE formation in kidney is an adaptive response to As(III) treatment that will participate, at least in part, in improving renal function. The relative increase in hepatic 20-HETE formation could be attributed to the increased mRNA expression of Cyp1b1, Cyp4a10, Cyp4a14, Cyp4f13, Cyp4f15, Cyp4f16 and Cyp4f18, and Cyp4a protein expression in As(III)-treated mice. In the kidney however As(III) decreased 20-HETE formation could be attributed to the lower Cyp4a protein expression despite the elevated mRNA levels.

In conclusion, acute As(III) treatment alters the expression of several CYPs and sEH enzymes in an organ-specific manner. There are several mechanisms that can explain the effect of As(III) treatment on these enzymes. Although the exact mechanism is not fully elucidated yet, As(III)-induced inflammation and oxidative stress contribute, at least in part, in the alteration of the CYP enzymes and sEH. The changes in CYP and sEH expression result in altered arachidonic acid metabolism as 20-HETE formation was increased in the liver and decreased in the kidney. To the contrast, EETs formation was increased only in the kidney. Taking into account the physiological functions of these metabolites, the increase in EETs formation in addition to the decrease in 20-HETE in the kidney can be considered an adaptive response. Whereas, the increase of 20-HETE in the liver can be considered as a maladaptive response to As(III) treatment.

Declaration of Interest

The authors declare no conflicts of interests.

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant RGPIN 250139-07 to A.O.S. A.A.-M. is the recipient of Alberta Innovates Technology Futures Scholarship, and Izaak Walton Killam memorial graduate scholarship. A.A.E. is the recipient of Egyptian Government Scholarship. S.H.K. is the recipient of Alberta Innovates Health Solutions summer studentship. H.N.A. is the recipient of the Kingdom of Saudi Arabia government Scholarship. O.H.E. is the recipient of University of Alberta recruitment

scholarship. B.N.M.Z. is the recipient of Alberta Innovates Health Solutions Scholarship.

References

Aboutabl ME, Zordoky BN, El-Kadi AO. (2009). 3-methylcholanthrene and benzo(a)pyrene modulate cardiac cytochrome P450 gene expression and arachidonic acid metabolism in male Sprague Dawley rats. Br J Pharmacol 158:1808–19.

Anwar-Mohamed A, El-Sherbeni AA, Kim SH, et al. (2012). Acute arsenic toxicity alters cytochrome P450 and soluble epoxide hydrolase and their associated arachidonic acid metabolism in C57Bl/6 mouse heart. Xenobiotica 42:1235–47.

Anwar-Mohamed A, Zordoky BN, Aboutabl ME, El-Kadi AO. (2010). Alteration of cardiac cytochrome P450-mediated arachidonic acid metabolism in response to lipopolysaccharide-induced acute systemic inflammation. Pharmacol Res 61:410–18.

Baldwin WS, Roling JA. (2009). A concentration addition model for the activation of the constitutive androstane receptor by xenobiotic mixtures. Toxicol Sci 107:93–105.

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 69:1559–71.

Bhattacharyya KK, Brake PB, Eltom SE, et al. (1995). Identification of a rat adrenal cytochrome P450 active in polycyclic hydrocarbon metabolism as rat CYP1B1. Demonstration of a unique tissue-specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. J Biol Chem 270:11 595–602.

Birks EK, Bousamra M, Presberg K, et al. (1997). Human pulmonary arteries dilate to 20-HETE, an endogenous eicosanoid of lung tissue. Am J Physiol 272:L823–9.

Choudhary D, Jansson I, Stoilov I, et al. (2004). Metabolism of retinoids and arachidonic acid by human and mouse cytochrome P450 1b1. Drug Metab Dispos 32:840–7.

Danielson PB. (2002). The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 3:561–97.

Das J, Ghosh J, Manna P, et al. (2009). Taurine protects rat testes against NaAsO(2)-induced oxidative stress and apoptosis via mitochondrial dependent and independent pathways. Toxicol Lett 187:201–10.

Davey JC, Nomikos AP, Wungjiranirun M, et al. (2008). Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor-and thyroid hormone receptor-mediated gene regulation and thyroid hormone-mediated amphibian tail metamorphosis. Environ Health Perspect 116:165–72.

Elbekai RH, El-Kadi AO. (2006). Cytochrome P450 enzymes: central players in cardiovascular health and disease. Pharmacol Ther 112:564–87.

Enayetallah AE, French RA, Thibodeau MS, Grant DF. (2004). Distribution of soluble epoxide hydrolase and of cytochrome P450 2C8, 2C9, and 2J2 in human tissues. J Histochem Cytochem 52:447–54.

Felix K, Manna SK, Wise K, et al. (2005). Low levels of arsenite activates nuclear factor-kappaB and activator protein-1 in immortalized mesencephalic cells. J Biochem Mol Toxicol 19:67–77.

Hao CM, Breyer MD. (2007). Physiologic and pathophysiologic roles of lipid mediators in the kidney. Kidney Int 71:1105–15.

Hoff U, Lukitsch I, Chaykovska L, et al. (2011). Inhibition of 20-HETE synthesis and action protects the kidney from ischemia/reperfusion injury. Kidney Int 79:57–65.

Imaoka S, Hashizume T, Funae, Y. (2005). Localization of rat cytochrome P450 in various tissues and comparison of arachidonic acid metabolism by rat P450 with that by human P450 orthologs. Drug Metab Pharmacokinet 20:478–84.

Imig JD, Navar LG, Roman RJ, et al. (1996). Actions of epoxygenase metabolites on the preglomerular vasculature. J Am Soc Nephrol 7:2364–70.

Kaspera R, Totah RA. (2009). Epoxyeicosatrienoic acids: formation, metabolism and potential role in tissue physiology and pathophysiology. Expert Opin Drug Metab Toxicol 5:757–71.

Kimura A, Ishida Y, Hayashi T, et al. (2006). Interferon-gamma plays protective roles in sodium arsenite-induced renal injury by up-regulating intrarenal multidrug resistance-associated protein 1 expression. Am J Pathol 169:1118–28.

Kohle C, Bock KW. (2009). Coordinate regulation of human drug-metabolizing enzymes, and conjugate transporters by the Ah receptor, pregnane X receptor and constitutive androstane receptor. Biochem Pharmacol 77:689–99.

Kumazaki M, Ando H, Sasaki A, et al. (2011). Protective effect of alpha-lipoic acid against arsenic trioxide-induced acute cardiac toxicity in rats. J Pharmacol Sci 115:244–8.

Liu J, Liu Y, Powell DA, et al. (2002). Multidrug-resistance mdr1a/1b double knockout mice are more sensitive than wild type mice to acute arsenic toxicity, with higher arsenic accumulation in tissues. Toxicology 170:55–62.

Loot AE, Fleming I. (2011). Cytochrome P450-derived epoxyeicosatrienoic acids and pulmonary hypertension: central role of transient receptor potential C6 channels. J Cardiovasc Pharmacol 57:140–7.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–75.

Maier KG, Roman RJ. (2001). Cytochrome P450 metabolites of arachidonic acid in the control of renal function. Curr Opin Nephrol Hypertens 10:81–7.

Malaplate-Armand C, Ferrari L, Masson C, et al. (2003). Astroglial CYP1B1 up-regulation in inflammatory/oxidative toxic conditions: IL-1beta effect and protection by N-acetylcysteine. Toxicol Lett 138:243–51.

Manimaran A, Sarkar SN, Sankar P. (2010). Influence of repeated preexposure to arsenic on acetaminophen-induced oxidative stress in liver of male rats. Food Chem Toxicol 48:605–10.

Marden NY, Fiala-Beer E, Xiang SH, Murray M. (2003). Role of activator protein-1 in the down-regulation of the human CYP2J2 gene in hypoxia. Biochem J 373:669–80.

Mathews V, Desire S, George B, et al. (2006). Hepatotoxicity profile of single agent arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia, its impact on clinical outcome and the effect of genetic polymorphisms on the incidence of hepatotoxicity. Leukemia 20:881–3.

Mazumder DN. (2005). Effect of chronic intake of arsenic-contaminated water on liver. Toxicol Appl Pharmacol 206:169–75.

Mitchell SR, Sewer MB, Kardar SS, Morgan ET. (2001). Characterization of CYP4A induction in rat liver by inflammatory stimuli: dependence on sex, strain, and inflammation-evoked hypophagia. Drug Metab Dispos 29:17–22.

Moreno C, Maier KG, Hoagland KM, et al. (2001). Abnormal pressure-natriuresis in hypertension: role of cytochrome P450 metabolites of arachidonic acid. Am J Hypertens 14:90S–7S.

Morisseau C, Hammock BD. (2007). Measurement of soluble epoxide hydrolase (sEH) activity. Curr Protocol Toxico 33:1–18.

Nilakantan V, Maenpaa C, Jia G, et al. (2008). 20-HETE-mediated cytotoxicity and apoptosis in ischemic kidney epithelial cells. Am J Physiol Renal Physiol 294:F562–70.

Patel HV, Kalia K. (2010). Sub-chronic arsenic exposure aggravates nephrotoxicity in experimental diabetic rats. Indian J Exp Biol 48:762–8.

Piscaglia F, Knittel T, Kobold D, et al. (1999). Cellular localization of hepatic cytochrome 1B1 expression and its regulation by aromatic hydrocarbons and inflammatory cytokines. Biochem Pharmacol 58:157–65.

Powell PK, Wolf I, Jin R, Lasker JM. (1998). Metabolism of arachidonic acid to 20-hydroxy-5,8,11, 14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. J Pharmacol Exp Ther 285:1327–36.

Qu W, Rippe RA, Ma J, et al. (1998). Nutritional status modulates rat liver cytochrome P450 arachidonic acid metabolism. Mol Pharmacol 54:504–13.

Rahman MM, Ng JC, Naidu R. (2009). Chronic exposure of arsenic via drinking water and its adverse health impacts on humans. Environ Geochem Health 31:189–200.

Ramanathan K, Shila S, Kumaran S, Panneerselvam C. (2003). Protective role of ascorbic acid and alpha-tocopherol on

arsenic-induced microsomal dysfunctions. Hum Exp Toxicol 22:129–36.

Roman RJ. (2002). P-450 metabolites of arachidonic acid in the control of cardiovascular function. Physiol Rev 82:131–85.

Sacerdoti D, Gatta A, Mcgiff JC. (2003). Role of cytochrome P450-dependent arachidonic acid metabolites in liver physiology and pathophysiology. Prostaglandins Other Lipid Mediat 72:51–71.

Schwartzman ML, Martasek P, Rios AR, et al. (1990). Cytochrome P450-dependent arachidonic acid metabolism in human kidney. Kidney Int 37:94–9.

Theken KN, Deng Y, Kannon MA, et al. (2011). Activation of the acute inflammatory response alters cytochrome P450 expression and eicosanoid metabolism. Drug Metab Dispos 39:22–9.

Umannova L, Zatloukalova J, Machala M, et al. (2007). Tumor necrosis factor-alpha modulates effects of aryl hydrocarbon receptor ligands on cell proliferation and expression of cytochrome P450 enzymes in rat liver ''stem-like'' cells. Toxicol Sci 99:79–89.

Wu JP, Chang LW, Yao HT, et al. (2009). Involvement of oxidative stress and activation of aryl hydrocarbon receptor in elevation of CYP1A1 expression and activity in lung cells and tissues by arsenic: an in vitro and in vivo study. Toxicol Sci 107:385–93.

Yanez L, Carrizales L, Zanatta MT, et al. (1991). Arsenic-cadmium interaction in rats: toxic effects in the heart and tissue metal shifts. Toxicology 67:227–34.

Yoshida S, Hirai A, Tamura Y, Yoshida S. (1990). Possible involvement of arachidonic acid metabolites of cytochrome P450 monooxygenase pathway in vasopressin-stimulated glycogenolysis in isolated rat hepatocytes. Arch Biochem Biophys 280:346–51.

Yu Z, Ng VY, Su P, et al. (2006). Induction of renal cytochrome P450 arachidonic acid epoxygenase activity by dietary gamma-linolenic acid. J Pharmacol Exp Ther 317:732–8.

Zhao X, Feng T, Chen H, et al. (2008). Arsenic trioxide-induced apoptosis in H9c2 cardiomyocytes: implications in cardiotoxicity. Basic Clin Pharmacol Toxicol 102:419–25.

Zordoky BN, Aboutabl ME, El-Kadi AO. (2008). Modulation of cytochrome P450 gene expression and arachidonic acid metabolism during isoproterenol-induced cardiac hypertrophy in rats. Drug Metab Dispos 36:2277–86.

Zordoky BN, Anwar-Mohamed A, Aboutabl ME, El-Kadi AO. (2010). Acute doxorubicin cardiotoxicity alters cardiac cytochrome P450 expression and arachidonic acid metabolism in rats. Toxicol Appl Pharmacol 242:38–46.