Regulation of CYP1A1 by heavy metals and consequences for drug metabolism

Anwar Anwar-Mohamed, Reem H Elbekai & Ayman OS El-Kadi†
†*University of Alberta, 3126 Dentistry/Pharmacy Centre, Edmonton, Alberta, T6G 2N8, Canada*

Cytochrome P450 1A1 (CYP1A1) is a hepatic and extrahepatic enzyme that is regulated by the aryl hydrocarbon receptor signaling pathway. With the growing human exposure to heavy metals, emerging evidence suggests that heavy metals exposure alter CYP1A1 enzyme activity. Heavy metals regulate CYP1A1 at different levels of its aryl hydrocarbon receptor signaling pathway in a metal- and species-dependent manner. The importance of CYP1A1 emerges from the fact that it has been always associated with the metabolism of pro-carcinogenic compounds to highly carcinogenic metabolites. However, recently CYP1A1 has gained status along with other cytochrome P450 enzymes in the metabolism of drugs and mediating drug–drug interactions. In addition, CYP1A1 has become a therapeutic tool for the bioactivation of prodrugs, particularly cytotoxic agents. In this review, we shed light on the effect of seven heavy metals, namely arsenic, mercury, lead, cadmium, chromium, copper and vanadium, on CYP1A1 and the consequences on drug metabolism.

Keywords: aryl hydrocarbon receptor, CYP1A1, drug metabolism, heavy metals

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1. Introduction

The aryl hydrocarbon receptor (AhR) is a member of basic-helix-loop-helix (bHLH)/Per-ARNT-Sim (PAS) family of transcription proteins. Inactive AhR resides in the cytoplasm bound to two 90-kDa heat-shock proteins (HSP90), the 23-kDa heat shock protein (p23) and hepatitis B virus X-associated protein 2 (XAP2). On ligand binding, the AhR-ligand complex dissociates from the cytoplasmic complex and translocates to the nucleus where it associates with the aryl hydrocarbon nuclear translocator (ARNT) [1]. The whole complex then acts as a transcription factor that binds to a specific DNA recognition sequence, termed the xenobiotic responsive element (XRE), located in the promoter region of a number of AhR-regulated genes. Among these genes are those encoding a number of drug metabolizing enzymes, including four Phase I enzymes (cytochrome P450 1A1 (CYP1A1), CYP1A2, CYP1B1 and CYP2S1) and four Phase II enzymes (NAD(P)H: quinone oxidoreductase-1 (NQO1), glutathione-*S*-transferase A1, cytosolic aldehyde dehydrogenase-3 and UDP-glucuronosyltransferase 1A6) [1,2]. In the present review, current knowledge about the effect of heavy metals, in particular, arsenic (As^{3+}), mercury (Hg^{2+}), lead (Pb^{2+}), cadmium (Cd^{2+}), chromium (Cr^{6+}), copper (Cu^{2+}) and vanadium (V^{5+}) on CYP1A1 is reviewed. In addition, its consequence on drug metabolism is discussed.

2. The AhR structure

The AhR protein contains several domains critical for its function. The bHLH motif located in the N-terminus of the protein contains two functionally distinctive

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and highly conserved domains [3]. The first is the basic region, which is mainly involved in the binding of AhR to DNA. The second is the helix-loop-helix domain, which is embroiled in protein–protein interactions. Members of the bHLH family also include *Drosophila* circadian rhythm protein period (Per), the ARNT and the *Drosophila* neurogenic protein single minded (Sim) [4-6]. The AhR protein contains two PAS domains, PAS-A and PAS-B. These PAS domains are involved in secondary interactions with other PAS containing proteins, such as AhR and ARNT [5,6]. Additionally, the PAS-B domain contains the AhR ligand binding site [7]. Finally, a glutamine (Q) rich region located within the C-terminal region was found to be responsible for transcriptional activation on AhR binding to the DNA [3].

Within the cytoplasm, HSP90s interact with the PAS-B and bHLH domains of the AhR, maintaining the high affinity ligand binding conformation of the AhR, and repressing its intrinsic DNA-binding activity [8]. The prostaglandin E synthase 3 (p23) is thought to stabilize the interaction between HSP90s and the AhR [9], protect the receptor from being degraded through proteolysis, and prevent the premature binding of AhR to ARNT. On the other hand, XAP2 interacts with the C-terminal of HSP90 and the nuclear localization sequence of the AhR, preventing inappropriate trafficking of the unliganded receptor to the nucleus [10,11].

3. CYP1A1 regulation

Among the CYP450s, CYP1A1 has received considerable attention because it is highly induced by a broad range of xenobiotics such as polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons (HAHs) through the AhR–XRE transcription pathway [12]. CYP1A1 is capable of producing polar, toxic or even carcinogenic metabolites from various AhR ligands, including PAHs. Dissection of the mammalian *CYP1A1* gene revealed several regulatory elements that modulate its expression. The first is the XRE, several of which are localized ~ 1 kb upstream of the transcription start site in all mammalian *CYP1A1* genes [13]. Second, there are three glucocorticoid responsive elements (GREs) in the human, rat and mouse C*YP1A1* gene in the first intron, responsible for the modulation of PAHs-induced CYP1A1 expression by glucocorticoids [14]. Last, the presence of a negative regulatory element, located between -560 and -831 bp, inhibits the constitutive expression of CYP1A1 due to an interaction with the nuclear transcription factor Oct-1 [13].

The regulation of CYP1A1 has been extensively studied, yet it is not completely understood. Changes in physiological conditions, including stressful conditions such as hyperoxia [15] and suspension of cells [16,17], or induction of differentiation [18], increase CYP1A1 expression in the absence of an exogenous ligand. With the identification of an active AhR in cell culture and tissue slices in the absence of an exogenous AhR ligand [19], it was proposed that a ligand-independent mechanism might be responsible for AhR activation and subsequent CYP1A1 induction [12]. Further proof of this theory arose from studies that showed that inhibition of nuclear export by leptomycin B or mutation of the AhR nuclear export sequence resulted in nuclear accumulation of AhR in the absence of an exogenous ligand [20]. These studies suggest that AhR shuttles between the nucleus and the cytosol in the absence of exogenous ligand. The exact mechanisms governing the ligand-independent activation of AhR are still not clear. However, it has been reported that activation of the cAMP mediator [21] or MAPK signaling pathways [22] increase AhR nuclear translocation.

Theories regarding ligand-independent activation of the AhR have been shadowed with the identification of a large number of endogenous compounds with the ability to activate the AhR *in vitro*. These ligands have been grouped into several categories, including indoles, tetrapyroles and arachidonic acid metabolites [12]. Indole-containing substances are primarily endogenous metabolites of tryptophan. Tryptophan and several of its naturally occurring metabolites, including tryptamine, indole acetic acid, indigo and indirubin, have been reported to activate the AhR in yeast and mammalian cell cultures [23,24]. Products of the heme degradation pathway have also been found to activate the AhR signaling pathway. Bilirubin and biliverdin activate the AhR in cultured cells at physiologically relevant concentrations [25,26]. Hydrophobic products of arachidonic acid metabolism, most notably lipoxin A4 and prostaglandins (PGs), also activate the AhR [27,28]. In fact, several PGs, including PGG2, PGD3 and PGH1, induced DNA binding of the AhR complex *in vitro* [28]. Interestingly, these endogenous ligands seem to be relatively weak inducers compared to the classic inducers (i.e., PAHs and HAHs).

The induction of Cyp1a1 by this structurally distinct class of compounds brings into light several identifiable factors that can influence the capacity of an AhR ligand to induce CYP1A1. AhR affinity plays a major role in determining the expression level of CYP1A1. This is illustrated in the C57BL/6 mice, which are sensitive to PAHs and HAHs [29], and the DBA/2 mice, which possess a lower affinity AhR and thus have lower induced Cyp1a1 levels (> 15-fold difference) [30]. Similarly, human beings also have > 12-fold variation in AhR affinity that was positively correlated to cancer incidents, particularly in cigarette smokers [31]. In addition to receptor affinity, the recruitment of different co-activators to the transcription complex also influences the capacity of the AhR to induce CYP1A1 expression [32]. It has been well documented that the chromatin core is comprised of a pair of histone proteins. Co-activators play an essential role in remodeling chromatin structure and relieving the transcription repressive effects of nucleosomes [32]. The AhR signaling pathway is modulated by several nuclear co-activators such as the CREB binding protein (CBP), p300, steroid receptor co-activators 1 and 2, receptor interacting protein 140, estrogen receptor-associated protein of 140 kD, silencing

mediator for retinoid acid and thyroid hormone receptors and ATPase-dependent chromatin remodeling factors such as Brahma-related gene 1 [33-40]. Studies examining the role of these co-activators in the regulation of AhR-regulated genes by heavy metals are in their infancy. Some are available that are included here.

4. AhR cross-talk with NRs

For the past couple of decades, extensive studies have been made to correlate the effect of different nuclear receptor (NR) inducers to the activation of AhR, which has been termed cross-talks. Of interest, there have been several attempts to explain these cross-talks. Generally, there are several theories explaining these cross-talks: that is, the competitive binding of different NRs to a DNA-binding site, selective dimerization with other NRs before the DNA-binding step and finally binding of different ligands that would probably affect the recruitment of a wide array of co-activators. Therefore, in the following section, we focus on four NRs cross-talks with AhR, namely, nuclear factor erythroid 2-related factor-2 (Nrf2), estrogen receptor (ER), glucocorticoid receptor (GR), retinoid activated receptors (RARs) and retinoid X receptors (RXRs).

4.1 Nrf2–AhR cross-talk

The XRE was identified to be the DNA motif responsible for the upregulation of a battery of genes, including Phase I and II drug metabolizing enzymes, by various AhR ligands (inducers). The antioxidant responsive element (ARE), which is the DNA motif responsible for the upregulation of particular Phase II genes, such as NQO1, glutathione-*S*-transferase A1, UDP-glucuronosyltransferase 1A6, aldehyde dehydrogenase-3 and heme oxygenase (HO-1), occurs primarily through the Nrf2/ARE signaling pathway. In fact, the increased gene expression of these enzymes in response to oxidative stress caused by agents such as isothiocyanate sulforaphane [41] and heavy metals occurs primarily through this signaling pathway [42]. Perturbation in the redox status of the cell activates Nrf2, a redox-sensitive member of the cap 'n' collar basic leucine zipper family of transcription factors [43]. Subsequently, Nrf2 dissociates from its cytoplasmic tethering polypeptide, Kelch-like ECH associating protein 1, and then translocates into the nucleus, dimerizes with a musculoaponeurotic fibrosarcoma protein, thereafter binds to and activates ARE [44]. The proximity of the CYP1A1 promoter (XRE) and ARE suggested a cross-talk and functional overlap between the two signaling pathways [45,46].

Recent reports suggest that bifunctional inducers, which activate both XRE and ARE signaling pathways, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), require direct cross-talk between the XRE- and ARE-mediated pathways for the induction of *NQO1* [46]. Furthermore, it has been reported that the induction of *NQO1* by ARE inducers requires the presence of AhR, suggesting a more direct cross-talk between the XRE- and ARE-mediated pathways [44,45,47]. Intriguingly, recent studies suggest that mouse Nrf2 is under the control of AhR, as evident by increased Nrf2 mRNA transcripts by AhR ligands [45]. Another study has demonstrated that the expression of AhR, Cyp1a1 and Cyp2b1 in Hepa 1c1c7 cells is partially dependent on Nrf2, implying that Nrf2 modulates both transcription of AhR and its downstream targets [48]. This was further supported by the reduction of AhR mRNA levels in Nrf2 knockout mice cells compared to wild type [48]. Furthermore, the AhR mRNA levels were increased in Kelch-like ECH associating protein 1 knockout mice cells, suggesting more direct role of Nrf2 in the regulation of AhR [48].

4.2 ER–AhR cross-talk

The cross-talk between ER and AhR has been previously reported [49]. In a study by Ohtake *et al.*, it was shown that the estrogenic action of AhR agonists could be exerted through a direct interaction between the AhR/ARNT complex on one side and the unliganded ER on the other side in the absence of 17β-estradiol [49]. This observation was further supported by the use of AhR and ER knockout mice in which 3-methyl-cholanthrene (3-MC) was unable to activate the estrogen-responsive genes, namely c-fos and VEGF, in both AhR and ER knockout mice [49]. Incongruously, Hoivik *et al.* found no effect of estrogen on the CYP1A1 induction in either Hepa 1c1c7 or human breast cancer MCF-7 cells [50].

Until now, no estrogen responsive elements in the CYP1A1 gene have been identified. However, a mutual inhibition between ERα and AhR for their corresponding response elements has been previously reported and is a matter of debate [51]. For example, there was no direct effect of estradiol on the AhR/XRE binding, whereas on the other hand, the ERα-mediated suppression of induced CYP1A1 was successfully reversed by both ER antagonist and by co-expression of nuclear factor I, a transcription factor that interacts with both AhR and ERα, suggesting a direct cross-talk between the two NRs through competing on a common transcription factor [52]. Moreover, it was shown that AhR and ERα compete for several other co-activators, such as receptor interacting protein 140, ER-associated protein of 140 kD, and silencing mediator for retinoid acid and thyroid hormone receptors [34,37]. Inversely, AhR–ligands were shown to downregulate ER-dependent gene expression in human MCF-7 cells and in rodent estrogen-responsive tissues [53]. In addition, TCDD was shown to inhibit the interaction of ERα with its ligand and response element [54,55]. Thus, these results suggest a potential competition between AhR and ERα for common co-activators [37,52].

The contradictory effects of estradiol on the AhR-regulated genes could be attributed to several factors that would determine the degree and the direction of response on exposure to estradiols such as species-specific effects, the concentrations

of estradiol used and last the cell line specific effects. The last factor would be in fact related to the changing levels of certain transcription factors or co-activators among different cell lines from the same species.

4.3 GR–AhR cross-talk

It has been demonstrated that the inducibility of CYP1A1 by different PAHs and HAHs, which are known to be potent AhR ligands, is potentiated by the action of GR [14]. It has been previously reported that the C*YP1A1* gene first intron contains three GREs [14]. Of interest, exon 1 is a non-coding region and the initiation codon of *CYP1A1* gene expression is located within exon 2 [14]. Thus, binding of ligand-activated GR to the GRE sequences in the first intron will interact with the initiation complex on the CYP1A1 promoter (XRE) and consequently enhances the level of induction of CYP1A1 enzyme by AhR–ligands transcriptionally [14]. Whereas on the other hand GR will not be able to initiate the transcription process alone.

In contrast, the AhR-regulated *CYP1B1* gene expression was suppressed by dexamethasone in fibroblasts through GR-dependent mechanism. Studies performed to explain this awkward response showed that this effect was mediated by a 256 bp DNA fragment carrying the XRE response element but not the GRE [56]. Thus, one may speculate that dexamethasone might act through a different transcriptional mechanism to downregulate *CYP1B1* gene expression. Furthermore, the modulation of both AhR-regulated genes (*CYP1A1* and *CYP1B1*) probably involves protein–protein interactions between the GR and other transcription factors, or by competing for a common co-activator [57].

4.4 RARs– and RXRs–AhR cross-talks

Retinoic acid (RA) has been identified as the most potent vitamin A metabolite that regulates a diverse group of physiological processes including but not limited to growth, differentiation, cell proliferation and morphogenesis [58]. The physiological effects of RA are mediated by a group of nuclear proteins RAR-α,β,γ and RXR-α,β,γ [59]. It is believed that RXRs are the master regulators because of the fact that they dimerize either with themselves to form homo- or hetero-dimers or with most of the nuclear transcription factors [60]. These receptor complexes then modulate the transcription of target genes through interacting with the DNA *cis*-acting RA response element [61].

The effect of RA on the regulation of CYP1A1 is contradictory. For example, studies carried on keratinocytes showed that RA was able to downregulate or upregulate CYP1A1 gene expression [60]. In a study by Vecchini *et al.* it was shown that keratinocytes CYP1A1 gene promoter contains an unusual RA response element [60]. Other studies on hepatocytes showed that RA had minimalist effect on CYP1A1 or CYP1A2 mRNAs, whereas RARs and RXRs selective ligands caused a pronounced decrease in hepatic CYP1A2 expression *in vivo* [62,63].

5. The role of CYP1A1 in mediating the toxic effects of PAHs and HAHs

CYP1A1 is not only regulated by the classical AhR ligands such as PAHs, but is also responsible for activating these compounds into highly toxic metabolites. Xenobiotics have also been shown to be activated by CYP1A1 into genotoxic and carcinogenic metabolites. Benzo[a]pyrene (BaP), the prototype carcinogenic PAH, is metabolized by CYP1A1 to BaP-7,8-oxide, which is subsequently hydrolyzed by epoxide hydrolase to (+)- and (-)-BaP-7,8-diol. CYP1A1 is then again responsible for the conversion of these metabolic intermediates into the toxic bay region metabolites, (-)-BaP-7,8diol-9,10-oxide-1, (+)-BaP-7,8-diol-9,10-oxide-2, (+)-BaP-7,8-diol-9,10-oxide-1 and (-)-BaP-7,8-diol-9,10-oxide-2 [64]. All four epoxides are highly mutagenic, yet (+)-BaP-7,8-diol-9,10-oxide-2 was identified as the most tumorigenic of all metabolites [65,66]. The toxicity of other bay region epoxide metabolites has similarly been reported for other PAHs, including benz[a]anthracene-3,4-diol, benz[b]fluoranthene-9,10-diol and benzo[c]phenanthrene-3,4-diol [64,66-68].

Other members of the CYP1 family are also involved in xenobiotic activation. CYP1B1 metabolizes PAHs, aryl- and heterocyclic amines to genotoxic metabolites [69]. In fact, Cyp1b1-null mice were not able to metabolize 7,12-DMBA and were resistant to the formation of lymphomas and other malignancies caused by 7,12-DMBA [70]. Furthermore, CYP1B1 is the principal precipitant of PAH-mediated immunotoxicity [71]. Interestingly, *in vitro* work demonstrated a 10-fold higher activity for CYP1B1 than CYP1A1 in converting BaP to BaP-7,8-diol in a reconstituted human enzyme system [72]. However, similar rates of activation were reported for CYP1A1 and CYP1B1 in the activation of various PAH diols, including (-)-BaP-7,8-diols, 7,12-DMBA-3,4-diol and B[a]A-3,4-diol [67,73]. On the other hand, CYP1A2 demonstrated different enzyme kinetics as it had slower rates in the activation of these diols [64].

6. Heavy metals and their effect on CYP1A1

Heavy metals are non-essential elements in that they are neither created nor biodegradable. They are mostly considered highly toxic agents that are introduced into the environment through natural and/or industrial resources [74]. Owing to their persistent occurrence and accumulation in the environment, and subsequent human exposure, the Agency for Toxic Substances and Diseases Registry [75] and the Canadian Environmental Protection Act Registry have ranked heavy metals among the most hazardous and toxic substances in the environment [76]. Among all heavy metals, As^{3+}, Hg^{2+}, Cr^{3+}, Pb^{2+} and Cd^{2+} are ranked the highest.

The persistence of heavy metals in the environment prompted the study of human exposure to mixtures of these heavy metals and AhR ligands in the environment and their biological response. Environmental co-contamination with

metals and AhR-ligands was discovered and reported worldwide. PAHs and heavy metals were found in airborne particulates in an industrial north Italian town, certain areas of Czech Republic where coal is used for heating and power [77,78], and in tissues of wild city pigeons exposed to air pollution in the Netherlands [79]. PAHs and heavy metals were also found in various aquatic sites such as the river Meuse in Western Europe [79], the Dniester River in the former Soviet Republic of Moldova [80], the New Bedford Harbor in Massachusetts [81] and in the Sheboygan River system in east-central Wisconsin [82].

With the growing human exposure to AhR ligands and heavy metals, emerging evidence suggests that AhR ligands and metal co-exposure generates biological responses different from what is expected based on the toxicological mechanisms of each class separately. Modulation of AhR ligand-dependent toxicities by heavy metals may occur by several mechanisms. One mechanism involves modulation of AhR ligand-induced CYP1A1 expression, a subject of intense interest in the scientific community.

6.1 As^{3+}

The first to report an effect of As^{3+} on CYP1A1 was Falkner *et al.* [83]. In this study, it was reported that As^{3+} alone inhibited the CYP1A1-dependent 7-ethoxyresorufin-O-deethylation activity in all tested tissues of guinea pig, but to a lesser extent in the lungs. Interestingly, however, As^{3+} potentiated the β-napthoflavone (βNF)-mediated induction of CYP1A1 activity in the lungs but decreased βNF-induced CYP1A1 activity in the kidneys and liver. In another study examining the effect of As^{3+} in Wistar rats, As^{3+} decreased total hepatic CYP450 content and monoxygenase activities of several CYP450s including CYP1A1 [84]. Early studies on primary cultures of chick hepatocytes showed that similar to the observations in the previous study, As^{3+} decreased total CYP450 and 3-MC-mediated induction of CYP1A1 activity [85]. In another study by the same group, using primary cultures of rat hepatocytes, As^{3+} decreased 3-MC-mediated induction of CYP1A1 mRNA, protein and catalytic activity levels [86]. More recently, the effect of several metals, including As^{3+}, was tested in the presence of several PAHs in primary human hepatocytes [87]. It was shown that As^{3+} decreased PAHs-mediated induction of CYP1A2 mRNA but not CYP1A1 mRNA levels in these cells, although both enzymes are regulated by AhR. Furthermore, As^{3+} decreased the PAHs-mediated induction of CYP1A1/1A2 protein and catalytic activity levels. Furthermore, studies from our laboratory have shown the ability of As^{3+} to inhibit Cyp1a1 activity in mouse Hepa 1c1c7 cells although potentiating mRNA and protein levels [88] in the presence of several AhR ligands.

Although the effect of As^{3+} on CYP1A1 activity does not always parallel its effect on the expression on CYP1A1 mRNA, almost all studies have reported a decrease in CYP1A1 activity in hepatic tissue or cells. Thus, several, but common, underlying pathways may be involved. Studies have shown that As^{3+}-dependent decrease in CYP1A1 activity was accompanied either by a decrease or no change in mRNA levels. Some studies have also reported a decrease in CYP1A1 activity in spite of an increase in mRNA levels. As^{3+} has also been shown to decrease the activity levels of other CYP450 enzymes not regulated by the AhR [89]. Thus, it seems that As^{3+} may have a direct effect on the function of the CYP450 protein, independent of transcriptional regulation. As such, it has been well documented that As^{3+} interacts with critical cysteine residues of many intercellular proteins, thus, altering their functions [90]. For example, As^{3+} has been shown to prevent the activation of NF-κB through interacting with cysteine 179 in the activation loop of the I-κB kinase catalytic subunit, and subsequently inhibiting the dissociation of I-κB from NF-κB, which is a necessity for NF-κB activation [91]. On the other hand, activity of CYP450s, which are heme-containing proteins, critically depends on the binding of heme iron to the sulfur atom of a conserved cysteine residue in the apoprotein [92]. It could, therefore, be speculated that As^{3+} can inhibit CYP1A1 catalytic activity by competing with heme for binding to the critical cysteine residue in the apoprotein [93]. Another possible mechanism for this observation is that metal-induced reactive oxygen species (ROS) may oxidize thiol groups in cysteine molecules, directly or indirectly through the formation of reactive nitrogen species, of the CYP1A1 protein causing loss of protein function [94]. ROS may also interact with the heme Fe^{2+} leading to heme destruction and enzyme inactivation [95].

HO-1, an enzyme of 32 kDa, catalyzes the oxidative conversion of heme into biliverdin, which serves an important role in protecting cells from oxidative damage, such as free radicals [96]. HO-1 is regulated through the redox sensitive Nrf2/ARE signaling pathway. Furthermore, HO-1 anchors to the endoplasmic reticulum membrane through a stretch of hydrophobic residues at the C-terminus [97]. Thus, it is expected to interact with CYP450s, which are also endoplasmic reticulum-bound enzymes.

As^{3+} has been shown to stimulate the production of superoxide (O_2^-) and hydrogen peroxide as a result of its intrinsic ability to accumulate in the mitochondria and alter cellular respiration. Investigating the role of oxidative stress in the regulation of Cyp1a1 by As^{3+} in Hepa 1c1c7 cells, we have shown that As^{3+} alone increased HO-1 mRNA that coincided with increased cellular glutathione (GSH) levels, either to compensate the oxidative stress production by As^{3+} or as a direct response to oxidative stress [98]. As^{3+} decreased the TCDD-mediated induction of Cyp1a1 activity, and this inhibition was further potentiated when GSH was depleted.

From the early studies by Falkner *et al.*, the inhibition of CYP1A1 activity was suspected to involve the degradation of its heme content through the induction of HO-1 [83]. HO-1 activity was also elevated in primary cultures of chick hepatocytes treated with As^{3+} [86]. In our studies, HO-1 mRNA was elevated in response to As^{3+} chemical insult, and

the total cellular heme content was decreased. However, we speculate that the induction of HO-1 may contribute to the inhibition of Cyp1a1 activity by the metals, and there must be other interplaying mechanisms. The effect of As^{3+} on CYP1A1 was not reversed by mesoporphyrin, an inhibitor for HO-1 [86]. Similarly, the addition of heme increased HO-1 activity to similar levels with or without As^{3+}, but the decrease in CYP1A1 activity was observed only when As^{3+} was present in primary cultures of rat hepatocytes [86]. Thus, the elevated levels of HO-1 alone may not be responsible for As^{3+}-mediated effects on CYP1A1. Therefore, there is a possibility that As^{3+} causes induction of HO-1 with a consequent decrease in the heme pool that could result in the failure to form a functioning protein. Thus, the apoprotein would be more susceptible to proteasomal degradation.

In contrast to some previously mentioned studies, it has been reported that As^{3+} may decrease CYP1A1 protein and activity levels. BaP-mediated induction of CYP1A1 mRNA in human breast cancer cells (T-47D) was not affected by As^{3+} treatment although BaP-mediated induction of CYP1A1 protein and catalytic activity levels were decreased on exposing the cells to As^{3+} [99]. This decrease in CYP1A1 activity coincided with an increase in HO-1 mRNA. In the absence of a decrease in CYP1A1 mRNA levels, As^{3+} may have decreased the CYP1A1 protein half-life through an increase in protein degradation. In our studies, however, we have shown that As^{3+} had no effect on Cyp1a1 protein stability [100].

The uncertainties in the mechanisms involved in the modulation of CYP1A1 activity and protein level by $As^{3+}$are also accompanied by many questions regarding the effect of this metal on mRNA levels. As^{3+} failed to attenuate the BaP-mediated induction of CYP1A1 mRNA, yet decreased its catalytic activity [101,102]. These results were in concordance with another study showing that pretreatment of human lung adenocarcinoma cells (CL3) with As^{3+} did not affect the BaP-mediated induction of CYP1A1 mRNA [103]. Further discrepancies arose with the emergence of data on the effect of As^{3+} on XRE-driven luciferase reporter gene in Hepa-1 cells. Maier *et al.* have shown that despite having no effect on TCDD-induced Cyp1a1 mRNA, As^{3+} decreased luciferase activity in cells transfected with the XRE-driven luciferase reporter gene [104]. The discrepancy between the effect of As^{3+} on Cyp1a1 mRNA and its effect on the XRE-driven luciferase reporter gene was not explained.

Later, Bessette *et al.* demonstrated that As^{3+} alone did not affect CYP1A1 mRNA but reduced the benzo[k]fluoranthene-induced levels on treatment with As^{3+} [105]. Similar to earlier reports, As^{3+} decreased XRE-driven luciferase reporter gene activation, the inhibition of TCDD-mediated induction of CYP1A1 mRNA, and XRE-dependent luciferase activity was also observed in human hepatoma Huh7 cells [106]. Furthermore, it was demonstrated that the actions of As^{3+} on blocking CYP1A1 induction by TCDD were primarily through altering CYP1A1 transcription, possibly through inhibiting the recruitment of polymerase II to the CYP1A1 promoter, which is independent of the regulatory mechanisms initiated by As^{3+}-induced cell arrest [107].

Later studies contradicted previously reported data. The ability of As^{3+} to induce AhR nuclear translocation in Hepa-1 cells, and in the same efficiency as TCDD, was confirmed [108]. The increase in nuclear accumulation of the AhR resulted in increased expression of Cyp1a1 mRNA when the cells were treated with As^{3+} alone, and potentiation of BaP-mediated induction of Cyp1a1 mRNA was further increased [108]. Thus, there seems to be species-specific differences in the effect of As^{3+} on CYP1A1 mRNA expression.

We have shown that As^{3+} alone is able to increase Cyp1a1 mRNA while potentiating the induction of Cyp1a1 mRNA mediated by AhR ligands in Hepa 1c1c7 cells [88]. Furthermore, the increase in mRNA levels translated to an increase in Cyp1a1 protein levels.

In more extensive studies, we demonstrated that As^{3+} increased Cyp1a1 mRNA in Hepa 1c1c7 cells in a time- and dose-dependent manner. The inducibility of Cyp1a1 mRNA by As^{3+} was completely abolished after the addition of the RNA polymerase inhibitor actinomycin-D (Act-D), implying a requirement of *de novo* RNA synthesis [82]. The transcriptional regulation of Cyp1a1 by As^{3+} was confirmed by the ability of As^{3+} to increase the XRE-dependent luciferase activity despite not increasing the AhR nuclear accumulation.

To confirm the role of the AhR in the regulation of Cyp1a1 by As^{3+}, cycloheximide (CHX), a protein synthesis inhibitor that inhibits a labile protein required for the proteolysis of the AhR [109], intensified the effect of As^{3+} on Cyp1a1 transcription [110]. Moreover, MG-132, a 26-proteasome inhibitor that stabilizes the AhR protein, also intensified the effect of As^{3+} on Cyp1a1 transcription. As^{3+} also increased the stability of TCDD-induced Cyp1a1 mRNA transcripts. We concluded that an increase in transcription, through an AhR-dependent mechanism, and stabilization of mRNA are responsible for the detected increase in constitutive Cyp1a1 expression and the potentiation of TCDD-mediated induction of Cyp1a1 mRNA.

The mechanisms by which As^{3+} induces CYP1A1 mRNA transcription through the AhR remain unresolved. Heavy metals may alter some cellular metabolic pathways leading to the enhanced production of endogenous AhR ligands. The first of these endogenous ligands are the heme degradation products bilirubin and biliverdin. The induction of HO-1 by As^{3+} will result in the formation of biliverdin and subsequently bilirubin, which have been shown to act as AhR ligands [12,25,26,89]. However, although the increase in pulmonary CYP1A1 expression was associated with an increase in total plasma bilirubin concentrations, the administration of bilirubin, a possible endogenous ligand, to the lung through intra-tracheal injection did not increase CYP1A1 mRNA [111,112]. As^{3+}-induced oxidative stress may result in the subsequent release of arachidonic acid from glycerolphospholipids,

which has also been previously shown to regulate the AhR signaling pathway [12,27]. Moreover, As^{3+} might bind to vicinal thiols in HSP90, disrupting the molecular interaction between AhR and XAP2, which will subsequently cause the AhR translocation [108].

Another postulation implicates post-transcriptional modifications of histones in the increase in gene transcription. As^{3+} induces phosphorylation [113,114] and acetylation [113] of histone H3 through the extracellular signal-regulated kinase (ERK), p38 and the Akt1 pathways. Recently, histone deacetylation has been shown to be involved in the inducibility of Cyp1a1 mRNA transcription in human and mouse hepatoma cells [115] and HeLa cells [116].

Although many possible mechanisms by which As^{3+} induces Cyp1a1 mRNA expression in mouse hepatoma cells have emerged, much less evidence is available that may explain the discrepancy in the effect of As^{3+} on CYP1A1 expression in various species. It is expected that differing regulatory mechanisms may be involved in the regulation of the AhR-mediated induction of CYP1A1 in humans. Thus, it is not surprising that As^{3+} produces a differential effect on Cyp1a1 expression in mice.

6.2 Hg^{2+}

Hg^{2+} has been shown to be a contaminant of major concern in aquatic toxicology studies owing to its ubiquity in different species of fish [117-122]. The problem is acute in fish populating waters close to shores. For example, whales near the shores tend to have more Hg^{2+} in their skin compared to those in the mid-Pacific thousands of kilometers from land (unpublished data) [123].

The most toxic form of Hg^{2+} is the organic form, and yet almost all studies concerning its effect on the CYP1A1 are conducted using the inorganic form. The first report about the effect of Hg^{2+} on CYP1A1 was by Vakharia *et al.* [87]. In this study, it was shown that Hg^{2+} was the most potent inhibitor of CYP1A2 activity in freshly isolated human hepatocytes, and yet it was less potent than As^{3+} in inhibiting AhR ligand-mediated induction of CYP1A1 activity. This inhibition of PAH-mediated induction of CYP1A1 activity by Hg^{2+} was accompanied with a decrease in its protein levels [87]. However, at 5 µM, Hg^{2+} failed to decrease the BaP, benzo[b]fluoranthene, benzo[a]anthracene and benzo[k]fluoranthene-mediated induction of CYP1A1 activity and benzo[k]fluoranthene-mediated induction of CYP1A1 mRNA. It did, however, cause a small but significant decrease in dibenzo[a,h]anthracene-mediated CYP1A1 activity induction [102]. Interestingly, in the same study, Hg^{2+} potentiated the induction of CYP1A1 protein by benzo[k]fluoranthene. Thus, the effect of Hg^{2+} on CYP1A1 expression seems to be AhR-ligand specific.

In aquatic species, Hg^{2+} increased CYP1A1 protein levels in shark rectal gland cells, but the activity levels were not measured [124]. In hepatic sea bass (*Dicentrachus labrax* L.) microsomes, however, Hg^{2+} significantly decreased the βNF-mediated induction of CYP1A1 activity, which was partially reversed by GSH, implicating an ROS-dependent mechanism in the regulation of CYP1A1 by Hg^{2+} [125].

Experiments from our laboratory have shown that Hg^{2+} alone was able to increase Cyp1a1 mRNA without affecting its protein or activity levels in Hepa 1c1c7 cells. In contrast, using different AhR ligands, we have shown that Hg^{2+} potentiated the AhR ligand-mediated induction of Cyp1a1 mRNA and protein levels although decreasing its activity levels. An increase in protein half-life accounted for the increase in Cyp1a1 protein levels. However, the decrease in activity levels could only be accounted for by a decrease in heme content [126].

Hg^{2+} alone increased Cyp1a1 mRNA in a time-dependent manner while potentiating the TCDD-mediated induction of Cyp1a1 mRNA in a time- and TCDD concentration-dependent manner [127]. Also, Act-D completely blocked the Hg^{2+}-mediated induction of Cyp1a1 mRNA, implying requirement of *de novo* RNA synthesis. In the presence of CHX and/or MG-132, Hg^{2+}-mediated effect on Cyp1a1 mRNA was further potentiated suggesting that the Hg^{2+}-mediated induction of Cyp1a1 mRNA is transcriptional and AhR-dependent. In addition, Hg^{2+} was able to transform guinea-pig hepatic cytosol AhR, and induced nuclear accumulation of transformed AhR in Hepa 1c1c7 cells [127]. Contrary to the effect of As^{3+}, Hg^{2+} had no effect on Cyp1a1 mRNA transcript half-life. Thus, it is apparent that Hg^{2+} increases Cyp1a1 mRNA solely through a transcriptional mechanism that is dependent on AhR function. The cell-specific response to Hg^{2+} was clearly demonstrated when the HepG2 cells were used instead of Hepa 1c1c7 cells [128], as the TCDD-mediated induction of CYP1A1 protein and catalytic activity were decreased in this cell line in response to Hg^{2+} treatment [128]. Initial studies by Perdew *et al.* provided distinctive differences in the properties of unliganded human and mouse AhR such as cellular localization, nuclear translocation and the effects of XAP on both processes [129,130]. Different mechanisms have been offered to explain this phenomenon recently by Suzuki and Nohara [131]. Factors that could be responsible for these species-specific characteristics of AhR functions could be summarized in three major components: the nuclear translocation, transcription initiation through remodeling of chromatin and finally proteasomal degradation of AhR. In this study, it was shown that in Hepa 1c1c7 cells TCDD treatment causes the recruitment of co-activator CBP but not p300 to the CYP1A1 promoter region [131]. On the other hand, in HepG2, TCDD treatment caused the recruitment of p300 but not CBP to the CYP1A1 promoter region [131]. Thus, we may speculate that although TCDD is a potent AhR ligand and CYP1A1 inducer, it might be still functionally different across species. Another important difference between Hepa 1c1c7 and HepG2 that might participate in the species-specific responses is the sensitivity of AhR to proteasomal degradation on exposure to TCDD,

with the HepG2 AhR being less sensitive than the Hepa 1c1c7 AhR.

To determine the mechanism by which Hg^{2+} induces AhR translocation and induces Cyp1a1 transcription, we tested the role of the redox-sensitive transcription factors, NF-κB and activator protein-1 (AP-1) in the modulation of the Cyp1a1 gene by Hg^{2+} [132]. Both transcription factors are activated by changes in the redox status of cells and we have shown the induction of such environment in Hepa 1c1c7 cells by Hg^{2+}. In addition, previous reports have shown a mutual inhibitory interaction between the AhR and NF-κB and attenuation of AP-1 DNA binding by TCDD in an AhR-dependent manner [133,134]. Other studies have shown that these transcription factors share a number of co-activators, providing an explanation to this phenomenon [135-137].

To determine the role of NF-κB and AP-1 in the modulation of the Cyp1a1 gene, we first tested the ability of Hg^{2+} to activate these transcription factors. Not surprisingly, Hg^{2+} increased NF-κB and AP-1 binding to κB-RE and 12-O-tetradecanoylphorbol 13-acetate-responsive element as evidenced by electrophoretic mobility shift assay. Next, several activators and inhibitors of NF-κB and AP-1 were utilized to confirm their role in the modulation of Cyp1a1 by Hg^{2+}. The NF-κB activator phorbol 12-myrisitate 13-acetate (PMA) decreased Hg^{2+}-induced HO-1 mRNA and Cyp1a1 mRNA and activity. On the other hand, the NF-κB inhibitor pyrrolidinedithiocarbamate (PDTC) potentiated the induction of HO-1 mRNA and Cyp1a1 mRNA and activity in the presence of Hg^{2+}, implying a crucial role for NF-κB in the regulation of Cyp1a1 by Hg^{2+}.

The use of different inhibitors of AP-1 upstream regulators, such as the JNK inhibitor, SB600125, and the P38 inhibitor, SB203580, had similar effects to those of the NF-κB activator. On the other hand, the ERK inhibitor, U0126, had similar effect to those of NF-κB inhibitor. Altogether, these results imply that AP-1 through affecting its upstream signaling pathway might also be a key player in the regulation of Cyp1a1 by Hg^{2+}. Furthermore, these results imply the requirement of NF-κB and/or MAPK [138] and subsequently AP-1 in the regulation of Cyp1a1.

6.3 Pb^{2+}

The effect of Pb^{2+} on CYP1A1 was first tested in HepG2 cells [102]. In this study, it was shown that Pb^{2+} decreases CYP1A1 activity in the absence and presence of different PAHs without significantly affecting CYP1A1 mRNA levels [102]. In primary human hepatocytes, similarly, a decrease in CYP1A1 protein levels was associated with the decrease in CYP1A1 activity with Pb^{2+} treatment [87].

Our laboratory confirmed the results of Vakharia *et al.* [102]. We found that TCDD-induced CYP1A1 activity levels were reduced in the presence of Pb^{2+} compared to that of TCDD alone. The decrease in activity was also accompanied by a decrease in the protein expression levels [132].

However, using Hepa 1c1c7 cells, we have shown that similar to the effects observed with As^{3+} and Hg^{2+}, Pb^{2+} decreased Cyp1a1 activity but increased Cyp1a1 protein stability. Pb^{2+} also induced HO-1 mRNA levels, albeit with a lower intensity than As^{3+} and Hg^{2+}, and decreased the total cellular heme content, suggesting the involvement of post-transcriptional and post-translational mechanisms in the modulation of Cyp1a1 activity by Pb^{2+} [127].

Pb^{2+} alone was able to increase Cyp1a1 mRNA levels without affecting its protein levels. When different AhR ligands were co-administered with Pb^{2+}, the AhR ligand-mediated induction of Cyp1a1 activity was reduced in a dose- and AhR ligand-dependent manner [126]. In contrast, Pb^{2+} potentiated the TCDD-mediated induction of Cyp1a1 mRNA in a dose- and time-dependent manner [127]. Act-D completely abolished the Pb^{2+}-mediated induction of Cyp1a1 mRNA, implying a requirement for *de novo* RNA synthesis. AhR also seems to have a predominant role in this induction as co-treatment of Hepa 1c1c7 cells with CHX and/or MG-132 and Pb^{2+} potentiated the Pb^{2+}-mediated induction of Cyp1a1 mRNA. Pb^{2+} was also able to induce AhR protein transformation in untreated guinea-pig hepatic cytosol, and nuclear accumulation of transformed AhR in Hepa 1c1c7 cells. Similar to the effect of Hg^{2+}, Pb^{2+} did not alter Cyp1a1 mRNA half-life, indicating a predominant transcriptional role for the modulation of Cyp1a1 mRNA by this metal.

The role of the redox-sensitive transcription factors, NF-κB and AP-1, in the modulation of *Cyp1a1* gene expression by Pb^{2+} was also investigated [132]. We first confirmed the role of induction of oxidative stress by Pb^{2+} and its effect on Cyp1a1 expression. We found that Pb^{2+} was able to increase ROS production and decrease total cellular GSH content in Hepa 1c1c7 cells. The pro-oxidant buthionine-(S,R)-sulfoximine (BSO) potentiated Pb^{2+}-mediated induction of HO-1 and Cyp1a1 mRNA levels, although further suppressing the TCDD-mediated induction of Cyp1a1 activity by Pb^{2+}. In turn, Pb^{2+} was able to increase the binding of NF-κB and AP-1 to their corresponding response elements. In addition, the NF-κB activator, PMA, decreased the Pb^{2+}-mediated induction of both HO-1 and Cyp1a1 mRNAs, but further potentiated the suppressive effect of Pb^{2+} on TCDD-mediated induction of Cyp1a1 activity. In contrast, the NF-κB inhibitor, PDTC, potentiated the Pb^{2+}-mediated induction of HO-1 mRNA but inhibited its induction of Cyp1a1 mRNA. Moreover, the Pb^{2+}-mediated suppressive effect on the TCDD-mediated induction of Cyp1a1 activity was reversed.

The controversy between these observations is worth studying to dissect the exact mechanisms. The use of pharmacological inhibitors might not be the ultimately correct approach in mechanistic studies as there is no proof that these inhibitors, even claimed specific, will not interfere with other signaling pathways. Thus, the best approaches identified until now are the use of either deficient cell lines

or siRNAs to knock-down certain genes to confirm their roles in the signaling pathway of interest [139].

6.4 Cd^{2+}

Numerous studies have shown that Cd^{2+} exposure affects total hepatic CYP450 and monoxygenase activities in different mammalian systems [140-143]. Cd^{2+} significantly increased the CYP1A1, but not CYP1A2, activity in HepG2 [144,145]. However, CYP1A1 was not detected in the livers or kidneys of smokers or non-smokers exposed to Cd^{2+} [145]. In the presence of inducers, Cd^{2+} selectively inhibited dibenzo[a,h]anthracene-mediated induction of CYP1A1 activity, but had no effect on its mRNA levels in HepG2 cells [102]. Similarly, in primary human hepatocytes, Cd^{2+} reduced PAH-mediated induction of CYP1A1 protein and activity, but not mRNA, in a PAH-dependent manner [87]. However, Cd^{2+} was able to induce AhR-responsive luciferase expression in HepG2 cells [144]. Surprisingly, in human fibroblast cells (GM0637 and GM4429), Cd^{2+} increased CYP1A1 mRNA and activity and potentiated the toxicity of BaP [146].

In another study, Cd^{2+} increased hepatic CYP1A1 activity in male Sprague-Dawley rats [147], but decreased total intestinal CYP450 and CYP1A1 activities, but not hepatic CYP450 [148]. In mouse Hepa-1 cells, Cd^{2+} had no effects on Cyp1a1 mRNA in the absence and presence of TCDD, and was unable to alter XRE-dependent luciferase activity [104]. In Antarctic fish (*Trematomus bernacchii*) livers, Cd^{2+} completely suppressed BaP-mediated induction of CYP1A1 mRNA, protein and catalytic activity levels [149]. A similar effect was seen in primary hepatocytes of black seabream and rainbow trout and top minnow hepatoma cell line treated with 3-MC [150,151].

Perhaps the most interesting findings with regard to the effect of Cd^{2+} on Cyp1a1 are those published by our laboratory group [82]. We have demonstrated that Cd^{2+} alone was able to induce Cyp1a1 mRNA although potentiating AhR ligand-induced Cyp1a1 mRNA and protein in a ligand- and dose-dependent manner in Hepa 1c1c7 cells. Cd^{2+} also induced Cyp1a1 mRNA in a time- and dose-dependent manner. The transcription inhibitor, Act-D, completely blocked Cd^{2+}-mediated induction of Cyp1a1 mRNA implying requirement of *de novo* RNA synthesis. Cd^{2+} was also shown to be a potent inducer of AhR nuclear accumulation with a subsequent increase in the XRE-dependent luciferase activity. Cd^{2+} did not affect Cyp1a1 mRNA stability, indicating a predominant transcriptional mechanism for the increase in Cyp1a1 mRNA. In determining the role of oxidative stress in the modulation of Cyp1a1 by Cd^{2+}, we observed that pretreatment of Hepa 1c1c7 cells with the pro-oxidant BSO potentiated the Cd^{2+}-mediated induction of Cyp1a1 and HO-1 mRNA levels in the presence and absence of TCDD. *N*-acetylcysteine (NAC), on the other hand, protected against Cd^{2+}-induced modulation of Cyp1a1 mRNA.

On the contrary, although Cd^{2+} increased Cyp1a1 protein stability, it decreased AhR ligand-mediated induction of Cyp1a1 activity, which coincided with a potent increase in HO-1 mRNA and a decrease in total cellular GSH content [88]. This inhibition occurred simultaneously with an increase in HO-1 mRNA and a decrease in total cellular heme content. Interestingly, BSO also potentiated, although NAC protected against, the suppressive effect of Cd^{2+} on the TCDD-mediated induction of Cyp1a1 activity, further confirming the role of ROS in the inhibition of protein function.

6.5 Cr^{6+}

In contrast to other metals, much less information is available on the effect of Cr^{6+} on CYP1A1 expression and function. In different species of fish [125,152], Cr^{6+} decreased the hepatic CYP1A1 activity. The addition of GSH potentiated this inhibition, possibly by enhancing the reduction of Cr^{6+} to Cr^{5+}, Cr^{4+} and Cr^{3+}, and the subsequent production of ROS [125].

In more extensive work in Hepa-1 cells, it has been shown that Cr^{6+} decreases TCDD-mediated induction of Cyp1a1 mRNA, protein and activity in a dose-dependent manner [104]. Cr^{6+} also inhibited the XRE-dependent luciferase activity but failed to attenuate TCDD-induced nuclear accumulation of the AhR protein. Interestingly, although Cr^{6+} generates oxidative stress rapidly [153-155], it inhibited BaP-induced mutagenesis when it was co-administered with the AhR ligand, but not when administered before or after BaP [156]. Thus, the inhibition of Cyp1a1 expression may play a role in the reduction of BaP metabolism and subsequent DNA damage. Furthermore, the use of BSO or NAC did not affect the inhibition of XRE-dependent luciferase activity by Cr^{6+}, suggesting that a direct mechanism is involved in Cr^{6+}-mediated inhibition of XRE activation.

In our studies using Hepa 1c1c7 cells, we demonstrated that Cr^{6+}-induced constitutive Cyp1a1 mRNA levels in a dose- and time-dependent manner. The pro-oxidant BSO caused further potentiation to the Cr^{6+}-mediated induction of Cyp1a1 mRNA, although NAC protected against this induction. Thus, we concluded that ROS production by Cr^{6+} is a key player in Cyp1a mRNA induction. Furthermore, we proved that the increase in Cyp1a1 mRNA levels occurred mainly by an AhR-dependent transcriptional mechanism, and not by altering the stability of the mRNA transcripts.

Despite its induction of Cyp1a1 transcription, Cr^{6+} inhibited inducible Cyp1a1 mRNA expression. The inhibition of inducible but not constitutive gene expression by Cr^{6+} can be explained by its effect on histone deacetylase activity. Recently, Cr^{6+} was shown to inhibit BaP-mediated release of histone deacetylase-1 from chromatin, preventing the recruitment of the co-activator p300 to the Cyp1a1 transcriptional domain, hence, blocking the induction of gene transcription [157].

Similar to other metals, Cr^{6+} decreased AhR ligand-mediated induction of Cyp1a1 activity although causing an increase in Cyp1a1 protein stability [100]. Additionally, the ROS-mediated

mechanism was also involved because BSO and NAC potentiated, or protected against, the decrease in Cyp1a1 acitivity by Cr^{6+}.

6.6 Cu^{2+}

In the Antarctic fish (*T. bernacchii*) liver, Cu^{2+} significantly inhibited hepatic CYP1A1 activity and CYP1A protein content during co-exposures with BaP, but no effects were observed at the transcriptional level [149]. On the other hand, in striped bass fish (*Morone saxatilis*), the transcription of CYP1A1 was not affected by any tested Cu^{2+} concentration in any tissue [158]. In HepG2 cells, Cu^{2+} was able to increase CYP1A1 mRNA in a time- and dose-dependent manner [159] and potentiated the inducing effect of TCDD on CYP1A1 protein and catalytic activity levels [128]. Previous studies by Kim *et al.* [160] have reported that Cu^{2+} inhibited the hepatic microsomal CYP450 catalyzed reactions in mice and rats as a result of impaired NADPH-P450 reductase. In addition, it has been reported that Cu^{2+} deficiency increased CYP1A1 activity in rat small intestine [161], implying a suppressive effect of Cu^{2+} on CYP1A1.

In Hepa 1c1c7 cells, Cu^{2+}-induced constitutive Cyp1a1 mRNA, but only at low doses [126]. The transcription- and AhR-dependent requirements for Cu^{2+}-mediated effect on Cyp1a1 mRNA was confirmed by co-treatment of Hepa 1c1c7 cells with Act-D or CHX and/or MG-132. Intriguingly, Cu^{2+} successfully transformed untreated guinea-pig hepatic cytosol AhR protein although it failed to induce its nuclear accumulation in Hepa 1c1c7 cells as evident by cytosolic and nuclear electrophoretic mobility shift assays, respectively. Using different ligands, Cu^{2+} decreased the AhR ligand-mediated increase in Cyp1a1 mRNA in a dose- and ligand-dependent manner. Moreover, it was able to decrease the TCDD-mediated induction of Cyp1a1 mRNA in a time- and TCDD-dose-dependent manner but did not affect Cyp1a1 mRNA stability.

It was shown that Cu^{2+} increased the production of ROS that coincided with an increase in HO-1 mRNA and a decrease in cellular GSH content; thus, the role of the redox-sensitive transcription factors NF-κB and AP-1 in the regulation of Cyp1a1 gene by Cu^{2+} was also investigated [132]. Similar to what was seen with other metals, the binding of NF-κB and AP-1 to their corresponding responsive elements was also induced by Cu^{2+} treatment. The NF-κB activator PMA decreased Cu^{2+}-mediated induction of Cyp1a1 mRNAs although the NF-κB inhibitor PDTC caused a slight but significant increase in Cu^{2+}-mediated induction of Cyp1a1 mRNA. Thus, it is apparent that NF-κB plays a role in the regulation of Cyp1a1 expression by Cu^{2+}. The role of AP-1 is less clear as JNK inhibition decreased, but ERK inhibition further potentiated the Cu^{2+}-mediated induction of Cyp1a1 mRNA. Meanwhile, the inhibition of P38 had no effect. Thus, individual MAPKs, and not AP-1, have a role in the modulation of Cyp1a1 expression by Cu^{2+}.

It is worth mentioning that in this study, Cu^{2+} possessed the highest inhibitory potency at the activity level between Hg^{2+} and Pb^{2+}. Cu^{2+} decreased AhR ligand-mediated induction of Cyp1a1 protein and activity in a dose- and ligand-dependent manner [126]. Yet, at the post-translational level, Cu^{2+} was the most potent between Hg^{2+} and Pb^{2+} in increasing the Cyp1a1 protein half-life. Thus, the decrease in Cyp1a1 protein and subsequent decrease in activity is primarily caused by a decrease in AhR-ligand-induced mRNA levels. The pro-oxidant BSO did not affect HO-1 or Cyp1a1 mRNA levels although it potentiated the suppressive effect of Cu^{2+} on the TCDD-mediated induction of Cyp1a1 catalytic activity, implicating a minor, but direct, role for ROS in the inhibition of Cyp1a1 activity by this metal. In addition, the inhibition of NF-κB had no effect on the TCDD-mediated induction of Cyp1a1 activity, implying that NF-κB does not play a role in Cu^{2+}-mediated modulation of inducible Cyp1a1 expression. Similar to their effect on the Cyp1a1 mRNA levels, JNK inhibition potentiated, whereas ERK inhibition reversed, the suppressive effect of Cu^{2+} on the TCDD-mediated induction of Cyp1a1 activity. Thus, inhibition of Cyp1a1 mRNA and activity is mediated primarily through a JNK-dependent mechanism.

6.7 V^{5+}

Very little information is available on the effect of V^{5+} on the Cyp1a1 expression and function. We have demonstrated recently that V^{5+} was able to decrease the TCDD-mediated induction of Cyp1a1 mRNA, protein and catalytic activity levels and inhibit the TCDD-mediated induction of the XRE-dependent luciferase activity. This inhibition in XRE function coincided with a similar inhibition in the nuclear accumulation of the transformed AhR in Hepa 1c1c7 cells. Furthermore, the inhibition of AhR nuclear accumulation was not due to a decrease in its protein expression levels. Looking at the post-transcriptional and post-translational effects of V^{5+} on existing Cyp1a1 mRNA and protein levels, we showed that V^{5+} did not affect Cyp1a1 mRNA or protein stability. Our results also showed that V^{5+} did not significantly alter the HO-1 mRNA level or total cellular heme content. Thus, V^{5+} modulates Cyp1a1 function solely by inhibiting transcription in an AhR-dependent manner. The postulated effect of heavy metals on CYP1A1 expression is summarized in **Figure 1**.

7. CYP1A1 and drug metabolism

Unlike other metabolizing enzymes, CYP1A1 has been historically associated with the metabolism of pro-carcinogenic compounds to highly carcinogenic metabolites. As molecular mechanisms for the study of CYP450 function evolved, CYP1A1 has gained status along with other CYP450 enzymes in the metabolism of drugs and mediating drug–drug interactions. In addition, CYP1A1 has become a therapeutic tool for the bioactivation of prodrugs, particularly cytotoxic

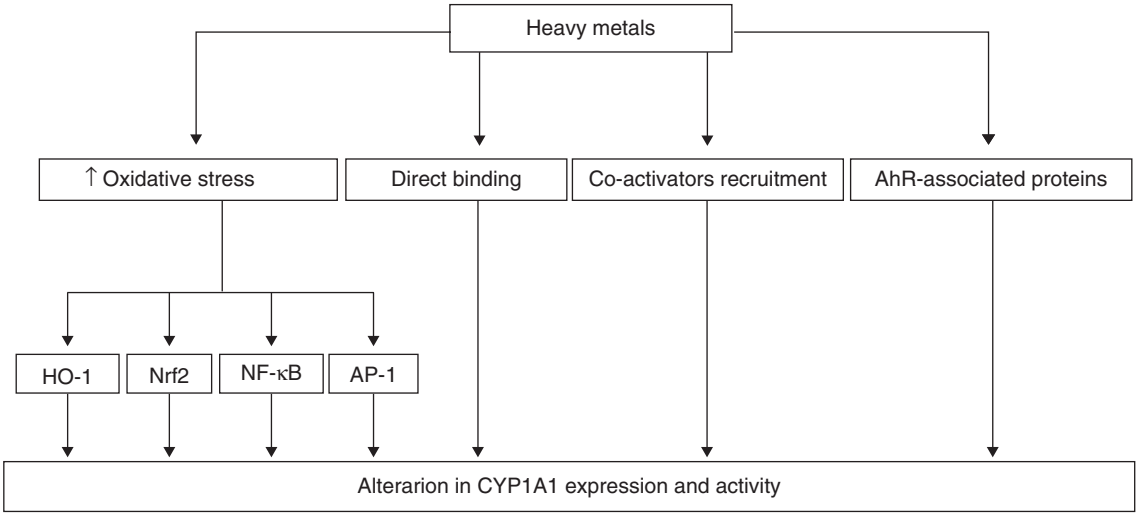

Figure 1. Proposed mechanisms by which heavy metals modulate CYP1A1 expression. Heavy metals modulate CYP1A1 expression through four main pathways. The first pathway involves induction of oxidative stress, which in turn will induce HO-1 that will in turn elevate heme catabolism. Also, oxidative stress will modulate Nrf2, NF-κB and AP-1 that will subsequently alter CYP1A1 expression. The second pathway involves direct interaction between heavy metals and CYP1A1 apoprotein, and subsequently alters CYP1A1 activity. The third pathway involves the alteration of recruitment of different co-activators required for the f CYP1A1 gene expression by heavy metals. Finally, heavy metals might alter the intercellular levels and activities of different AhR-associated proteins, such as HSP90, XAP2, p32 and ARNT.

AhR: Aryl hydrocarbon receptor; AP-1: Activator protein-1; ARNT: Aryl hydrocarbon nuclear translocator; CYP1A1: Cytochrome P450 1A1; HO-1: Heme oxygenase; HSP90: Heat-shock protein; Nrf2: Nuclear factor erythroid 2-related factor-2; XAP2: X-associated protein 2.

agents. Several agents, including widely used drugs, herbs and common dietary nutrients have been shown to be metabolized by CYP1A1 (**Table 1**).

Anticancer agents that are metabolized by CYP1A1 include the non-small cell lung cancer drugs gefitinib and erlotinib. Although both drugs are extensively metabolized by CYP3A4, patients often present with wide inter-individual variability in the pharmacokinetics of these two agents. A recent report has shown through *in vitro* metabolism studies that CYP1A1 exhibited higher maximum clearance of both drugs than CYP3A4 [162]. Interestingly, the AUC of erlotinib was found to be 2.8-fold lower in smokers [163]. Thus, CYP1A1 expression was considered a major determinant of gefitinib and erlotinib metabolism, exposure and efficacy.

CYP1A1 is not only important in determining exposure levels of anticancer agents, but it may also have a pivotal role in determining pharmacodynamic efficiency. 2-(4-Amino-3-methylphenyl)benzothiazole (DF 203) is a novel antitumor agent that has displayed potent activity towards breast, ovarian and colon cancer cells, yet the mechanism is still not understood [164]. Interestingly, this compound is metabolized by CYP1A1 to its inactive 6-hydroxy metabolite only in sensitive cells [164]. Moreover, DF 203 induces CYP1A1, and thus its own metabolism, only in sensitive cell lines [164]. Thus, it is postulated that CYP1A1 plays a yet unknown role in the antitumor activity of DF 203 [164]. The activation of another benzothiazole, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203), has also been shown to be dependent on CYP1A1 activity [165]. The dihydrochloride salt of 5F 203 (phortress) causes the induction of CYP1A1, which in turn metabolizes 5F 203 to electrophilic intermediates resulting in DNA damage and cell death [166].

CYP1A1 also serves an important role in the pharmacological efficacy of other anticancer drugs, including widely used therapeutics agents. Tamoxifen, the most common adjuvant therapy for the treatment of breast cancer is in fact a prodrug that is metabolized to the more potent 4-*trans*-hydroxytamoxifen metabolite [167]. Although CYP1A1 is a minor contributor to the bioactivation of tamoxifen, its role in determining the efficacy of this drug may be amplified in breast tumor, which has been shown to overexpress the CYP1A1 enzyme [168]. Another important anticancer agent metabolized by CYP1A1 is the DNA methylating agent dacarbazine. Dacarbazine is a broad range antitumor agent with activity against melanoma, Hodgkins's disease and sarcoma. Its reactive metabolites, N-demethylated species 5-[3-hydroxy-methyl-3-methyl-triazen-l-yl]-imidazole-4-carboxamide and 5-[3-methyl-triazen-1-yl]-imidazole-4-carboxamide, are formed following oxidative N-demethylation by CYP450 [169]. Although dacarbazine is metabolized by CYP1A2 and CYP2E1, CYP1A1 is the predominant enzyme responsible for its extrahepatic metabolism [169].

Many other anticancer agents have been reported to be metabolized by CYP1A1. Ellipticine, a DNA intercalating agent, is activated mainly by CYP3A4, followed by CYP1A1, CYP1A2, CYP1B1 and CYP2C9 [170]. Oltipraz, an

Table 1. Drugs metabolized by CYP1A1.

Drug	Pharmacological activity
5-fluorouracil [196]	Anticancer
AFP 464/aminoflavone [197]	Anticancer
Amiodarone [187]	Anti-arrhythmic
Bergamottin [198]	CYP3A4 inhibitor
Ciprofibrate/clofibrate [199]	Lipid regulating agent
Curcumin [200]	Oral cavity chemopreventive agent
Dexamethasone [201]	Corticosteroid
DF 203 [202]	Anticancer
Ellipticine [203]	Anticancer
Estradiol [204]	Estrogen (hormone replacement therapy)
Fluoroquinolones [205,206]	Anti-microbials
Flutamide [207]	Hormone antagonist
Fluvoxamine [208]	Selective serotonin re-uptake inhibitor
Ketocokonazole [209]	Anti-fungal
Lansoprazole [210]	Proton pump inhibitor
Norfluoxetine [208]	Selective serotonin re-uptake inhibitor
Oltipraz [211,212]	Anticancer
Omeprazole [213]	Proton pump inhibitor
Phenobarbital [214]	Anti-epileptic
Propioconozole [215]	Immunosuppressant (eczema and psoriasis)
Quinidine [191]	Anti-protozoal/anti-malarial
Quinine [191]	Anti-protozoal/anti-malarial
Resveretrol [216]	Inhibitor of AhR-induced carcinogenesis
Salicylamide [217]	Analgesic
Sertraline [208]	Anti-depressant
Sulindac [218]	NSAID
SYUIQ-5 [172]	Anticancer
Vitamin A [219]	Nutrient
Wy-14,643 [199]	Lipid regulating agent

AhR: Aryl hydrocarbon receptor; CYP1A1: Cytochrome P450 1A1.

anti-schistosomal agent, at present being evaluated as a chemopreventative agent, is metabolized by several CYP450 enzymes, including CYP1A1 [171]. Newer agents, including SYUIQ-5 [172] and AQ4N, are also metabolized by CYP1A1. Like many other chemotherapeutic agents, AQ4N is activated through metabolic processes. AQ4N, the di-N-oxide of 1,4-bis[{2-(dimethylaminoethyl}-amino]5,8-dihydroxyanthracene-9,10-dion, is reduced to AQ4 which binds DNA and inhibits topoisomerase activity. CYP1A1 and CYP2B6 have been cited as the most efficient enzymes in AQ4N reduction [173].

Interestingly, the metabolism of anticancer agents by CYP1A1 is being investigated as a therapeutic tool for targeted cytotoxic therapy. The induction of CYP1A1 in tumor tissue of solid cancers has been documented previously [174,175], but the levels of expression can vary among patients. New research into the targeting of tumor cells with genes encoding drug metabolizing enzymes is in its infancy.

In addition to anticancer agents, CYP1A1 has also been shown to be an important drug metabolizing enzymes for compounds with important physiological functions. Vitamin A and its derivatives (retinoids) play essential roles in embryogenesis and the regulation of inflammation, growth, apoptosis and differentiation. Retinoids have also been tagged for the treatment of various cancers and many skin diseases [176,177]. Retinol (vitamin A), the major retinoid absorbed, is metabolized by CYP1A1, in addition to CYP1A2, CYP1B1 and CYP3A4, to retinal [177]. CYP1A1 is also important for the biological activation of retinal, and its conversion to the active metabolite all-*trans*-retinoic acid (tretinoin), which exerts its effects through the binding of NRs. Furthermore, CYP1A1 plays an important role in determining the sensitivity or resistance to all-*trans*-retinoic acid because CYP1A1 has been shown to metabolize all-*trans*-retinoic acid to 18-hydroxy- and 4-hydroxy-retinoic acid [177].

Another therapeutic agent with physiological function is estradiol. Estradiol is used as a therapeutic agent in young women of childbearing age as a form of contraception and in postmenopausal women for the treatment of menopausal symptoms and the prevention of osteoporosis [178]. The importance of CYP1A1 in estradiol metabolism lies in understanding the effect of estradiol catabolism on homeostatic function and carcinogenesis.

Estradiol is metabolized by various CYP450 enzymes into many hydroxylated products. Although predominantely metabolized in the liver, CYP1A1 and CYP3A4 play a major role in the extra-hepatic conversion of estradiol to its 2-hydroxylated metabolites. 2-Hydroxyestradiol is further metabolized to 2-methoxyestradiol, a non-carcinogenic by-product with antiproliferative activity [178]. Although this CYP1A1 metabolite has been detected in breast tissue, the predominant pathway of estradiol metabolism in the breast and uterus is the formation of 4-hydroxyestradiol. This CYP1B1 metabolite is implicated in mediating the carcinogenic effect of estradiol owing to the generation of free radicals as it undergoes redox cycling [179]. Thus, it is plausible to predict that any effect on CYP1A1 activity may hinder or promote the carcinogenic effect of estradiol in target tissues.

CYP1A1 has also been shown to be a key enzyme in the oxidative metabolism of riluzole, a novel antiglutamate agent that has shown promise in the treatment of neurodegenerative diseases. CYP1A1-mediated N-hydroxylation and O-dealkylation of riluzole proceeds at a lower K_m and a higher biotransformation rate than the hepatic metabolism of this compound

by CYP1A2 [180]. CYP1A1 also plays a role in the metabolism of another neuroactive drug, granisetron [181]. Granisetron, a serotonin 5-HT3 receptor antagonist used to treat chemotherapy-induced nausea and vomiting, is metabolized primarily by CYP1A1 to 7- hydroxygranisetron and to a lesser degree to 9′-desmethylgranisetron by CYP1A1 and CYP3A4. Interestingly, a decade earlier, Bloomer *et al.* identified that both pathways of granisetron metabolism were inhibited by ketoconazole, probably through competitive inhibition [182]. Another antiemetic agent, ondansetron, is also metabolized by CYP1A1 [183]. In addition, chlorzoxazone, an approved muscle relaxant, is a CYP1A1 substrate [184].

Other drugs metabolized by CYP1A1 include those that play a role in cardiovascular health. Of great importance is the metabolism of R-warfarin by CYP1A1 (and CYP1A2). Warfarin is the most widely used anticoagulant. It is also one of the most difficult treatment regimens owing to its narrow therapeutic window and multi-drug and nutrient-drug interactions. Although less potent than its corresponding S-isomer, R-warfarin is also responsible for many significant drug interactions. CYP1A1 and CYP1A2 are both catalysts for the breakdown of R-warfarin to its 6-hydroxy and 8-hydroxy metabolites [185]. Thus, the level of expression of CYP1A1 in any one individual may serve as a source of variability and should be considered when adjusting doses in patients, particularly sporadic smokers.

As an effective anti-arrhythmic agent, amiodarone is widely used in the treatment of supraventricular and ventricular arrhythmias. Treatment with amiodarone is a source of several drug interactions, caused by the inhibition of several CYP450 enzymes by its primary metabolite, desethylamiodarone [186,187]. Although it also possesses anti-arrythmic activity, desethylamiodarone has also been shown to be more toxic [188]. Many early reports have shown CYP3A4 to be a major contributor to amiodarone metabolism, in addition to CYP2C8, CYP1A2, CYP2D6 and CYP2C19 [187]. Recently, however, human CYP1A1 was shown to possess higher affinity to amiodarone than CYP3A4 and a higher intrinsic clearance [189]. This information implicates the CYP1A1 in amiodarone toxicity drug interactions.

Another cardiovascular-active agent is pioglitazone, a thiazolidinedione antihyperglycemic metabolized by hydroxylation and oxidation by CYP2C8 and CYP3A4, and to a lesser degree by CYP1A1 (product monograph). Fluvastatin, a HMG CoA reductase inhibitor is mainly metabolized by CYP2C9 to 6-hydroxy- and N-deisopropyl-fluvastatin and 5-hydroxy-fluvastatin, with contribution from CYP1A1, which metabolizes the anti-cholesterol drug to its 5-hydroxy metabolite [190]. Debrisoquine, an investigational antihypertensive drug, has been historically used for genetic profiling of CYP2D6 polymorphism. Using recombinant CYP450s, it was discovered that debrisoquine 4-hydoxylation was carried out not only by CYP2D6, but also by CYP1A1. Furthermore, both the CYP2D6- and CYP1A1-mediated reactions were inhibited by quinidine and quinine [191].

Coumarin is another non-approved drug metabolized by CYP1A1 [192]. It is found in many plant species and has been used for many years as a fragrant in perfumes. Coumarin is also used in several countries for the treatment of lymphoedema [193]. However, it is metabolized by CYP1A1, CYP1A2 and CYP3A4 to a 3,4-epoxide responsible for the development of potentially lethal hepatotoxicity.

8. Expert opinion

As new pharmacological therapies enter the clinic, CYP1A1 will serve a greater role in mediating their therapeutic effect, toxic responses or drug interactions. The role of CYP1A1 in drug metabolism may ultimately be hampered by the presence of various agents and/or conditions that may alter the levels of CYP1A1 in the body.

As we have seen, heavy metals are potent inhibitors of CYP1A1 function. Environmental exposure to these contaminants may, thus, potentially result in altered pharmacokinetics, response or resistance to the therapeutic agents. Interestingly, As^{3+} (Trisenox™, Cephalon, Frazer, PA, USA) is successfully being used for the treatment of acute promyelocytic leukemia and multiple myeloma [194,195]. It is also being considered for treatment against solid tumors [195]. Drug–drug interactions between Trisenox and other agents have not been formally assessed. However, with what is known so far about the inhibition of CYP1A1 by As^{3+}, it is likely that this drug will alter the pharmacokinetics or pharmacodynamics of other compounds metabolized by CYP1A1, including other antitumor agents.

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Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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Affiliation

Anwar Anwar-Mohamed[1],
Reem H Elbekai[1] &
Ayman OS El-Kadi[†2] PhD
[†]Author for correspondence
[1]Faculty of Pharmacy
University of Alberta,
3126 Dentistry/Pharmacy Centre,
Edmonton, Alberta, T6G 2N8, Canada
[2]Associate Professor,
Faculty of Pharmacy
University of Alberta,
3126 Dentistry/Pharmacy Centre,
Edmonton, Alberta, Canada T6G 2N8, USA
Tel: +001 780 492 3071; Fax: +001 780 492 1217;
E-mail: aelkadi@pharmacy.ualberta.ca