Zuotai (β-HgS)-containing 70 Wei Zhen-Zhu-Wan (Rannasangpei) differs from mercury chloride and methylmercury on hepatic cytochrome P450

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Research

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Abstract

Background: Zuotai (mainly β-HgS)-containing 70 Wei-Zhen-Zhu-Wan (70W, Rannasangpei) is a famous Tibetan medicine for cardiovascular and gastrointestinal diseases. We have shown that 70W protected against CCl 4 hepatotoxicity. CCl 4 is metabolized via cytochrome P450 (CYP450) to produce reactive metabolites. Whether 70W has any effect on CYP450 is unknown and such effects should be compared with mercury compounds for safety evaluation.

Methods: Mice were given 70W (0.15-1.5 g/kg, po), Zuotai (30 mg/kg, po), HgCl 2 (33.6 mg/kg, po) and MeHg (3.1 mg/kg, po) for 7 days. Liver RNA and protein were isolated for qPCR and Western-blot analysis.

Results: 70W and Zuotai had no effects on hepatic Aryl hydrocarbon receptor (AhR) and CYP1A2, but HgCl 2 and MeHg increased Cyp1a2 mRNA and CYP1A2 protein levels;70W and Zuotai had no effects on constitutive androstane receptor (CAR) ,CYP2B andCYP2E1 expressions, but HgCl 2 increased CAR and Cyp2b10 mRNA, HgCl 2 and MeHg increased CYP2B and CYP2E1 protein expressions; 70W and mercury compounds had no apparent effects on the expression of pregnane X receptor (PXR) and Cyp3a11 mRNA, as well as CYP3A proteins. 70W and mercury compounds had no apparent effects on the expression of peroxisome proliferator-activated receptor alpha (PPARα) and CYP4A; but HgCl 2 tended to increase Cyp4a10 mRNA and CYP4A protein expressions. 70W and Zuotai had no apparent effects on the expression of farnesoid X receptor (FXR) and Cyp7a1 , while HgCl 2 and MeHg increased CYP7A1 expression.

Conclusions: Zuotai and Zuotai-containing 70W at clinical doses had minimal influence on hepatic CYPs, and the effects of 70W and Zuotai on CYP and corresponding nuclear receptors are different from HgCl 2 and MeHg.

Background

Tibetan Medicine is one of the important medical heritages of the world [1]. Zuotai, a Tibetan medicine mixture containing β-HgS, has been included in many famous Tibetan medicines in the treatment of a variety of diseases [2-4]. A systematic review of available studies of Tibetan medicine, however, indicates that the literature in Western industrialized countries is scarce [5]. The traditional Tibetan medicines use polyherbo-metallic mixture recipes, not a single ingredient in the treatment of diseases. For example, in a review of 193 herbo-metallic Tibetan medicines for liver diseases, herbs/plants (181 kinds), animal products (7 kinds), and minerals (5 kinds) were frequently used [6]. The well-designed pharmacology and clinical studies are encouraged to elucidate the pharmacological basis, safety, and clinical efficacy of Tibetan medicines [5,6].

We have recently reviewed the relevant literature and indicated that chemical compositions of minerals (metals) are a major determinant of their therapeutic effects and toxicity in Tibetan medicines [7]. 70W Zhen-Zhu Wan (70W, also called *Rannasangpei,* Pamda-28) is such an example [8]. 70W was developed

in the middle of fifteenth century and is composed of herbo-metallic mixtures, mainly from pearl, Hong-sik, Albergia odorifera, Nine stone, Saffron, Bezoar, Musk and Zuotai (a mineral mixture) in the treatment of cardiovascular diseases, gastrointestinal diseases, and neurodegenerative diseases [8], and is listed in the 2015 edition of Pharmacopoeia of China [9]. 70W is reported to be effective experimentally against vascular dementia in rats [10]. We have recently demonstrated that 70W is effective in protecting against LPS plus MPTP-induced chronic neuroinflammation and dopaminergic neuron loss, and ameliorated gut microbiota alterations [8]; 70W dose-dependently protected against CCl_4-induced liver injury, probably by inducing the Nrf2 antioxidant pathway [11].

CCl_4 is metabolized via cytochrome P450 (CYP450), particular CYP2E1, to produce reactive metabolites [12]. Whether the protective effects of 70W against CCl_4 hepatotoxicity is related to CYP450 inhibition is not known. In addition, since 70W has many beneficial effects as it contains enormous ingredients and it might be used in combination with other medications, the potential herb-drug interactions, especially on the liver CYP450 might occur. CYPs are the mixed function oxidase system mainly existing in the liver, and play roles in the metabolism of over 80% drugs [13]. Induction or inhibition of CYP450 is implicated in traditional medicine-induced hepatoprotection and/or hepatotoxicity [14-16]. CYP450 genes are regulated by corresponding nuclear receptors, their coordinate regulation affects hepatic phase I and phase II metabolisms [17].

Zuotai is a metal mixture, with 54% of β-HgS [18], and is used in a small amount as additions to many valuable Tibetan medicines [19]. 70W contains 5.8% of Zuotai [2,3 8, 18]. Hg is a toxic metal; the safety of Hg-containing traditional medicines is of concern [20]. Is Hg in Tibetan medicines really toxic? A recent human study revealed that Zuotai-containing Tibetan medicines are safe at clinical doses [19, 21, 22], including 70W [23]. Indeed, Zuotai differs from $HgCl_2$ and MeHg in producing hepatotoxicity [24], nephrotoxicity [25, 26], and intestinal toxicity with gut microbiome disruptions [27]. Whether Zuotai-containing 70W has any effect on CYP450 in comparison with mercury compounds is worth of investigation.

This study was therefore designed using 1-5 fold clinical doses of 70W (0.15, 0.5 and 1.5 g/kg, po) for oral administration for 7 days and compared its effects with equivalent Hg contents of Zuotai, $HgCl_2$, and 1/10 Hg contents of MeHg, in an attempt to obtain information for the safely use of Zuotai-containing 70W in the clinic.

Methods

Reagents

70W and Zuotai was provided by Tibetan Medicine Manufacture Factory as described previously [8, 11, 27], based on the 2015 edition of Pharmacopoeia of China for QA/QC control (Lot number

Z20110561).70W was prepared by grinding the pill into powder, adding distilled water to prepare the suspension for oral administration. Mercury chloride ($HgCl_2$) and methylmercury (MeHgCl) were from Sigma (St. Louis, MO, USA). All other chemicals were commercially available reagents.

Animals

Male Kunming mice were purchased from Animal Experimental Center of Third Military Medical University (Chongqing,China). Animals were maintained in the SPF-grade facilities at Zunyi Medical University, with controlled environment (22 ± 1°C, 50 ± 2% humidity and a 12 h: 12 h light: dark cycle) and free access to purified water and standard laboratory chow. All animal care and experimental protocols are complied with the Animal Management Guidelines of the Chinese Ministry of Health and approved by Animal Use and Care Committee of Zunyi Medical University (2013-05).

Animal treatments

Mice were randomly divided into 7 groups, respectively as Control, 70W (0.15, 0.5, 1.5g/kg), Zuotai (30 mg/kg, the amount contained in 70W), $HgCl_2$ (33.6 mg/kg, equivalent Hg as HgS) and MeHgCl (MeHg, 3.1 mg/kg, 1/10 of Hg). Mice were given oral administration for consecutive 7 days. The dose regimen selection was based on our prior publications [8, 11, 24-27]. Livers were collected 24 h after the last dose and stored at -80°C prior to analysis.

Liver toxicity evaluation

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by commercial kits according to the manufacturer' protocol. Liver samples were fixed in 10% formalin prior to routine processing and paraffin embedding. Liver sections (4 μm) were stained with hematoxylin and eosin and evaluated for hepatocellular lesions

Real-time PCR

Approximately 50-100 mg of tissues was homogenized in 1 ml TRIzol (TakaRa Biotechnology, Dalian, China) and total RNA was extracted according to manufacturer's instructions. The quality and quantity of RNA were determined by the Nanodrop (Thermo Scientific, ND-2000, USA), with 260/280 ratio >1.8. Total RNA was reverse transcribed with a High Capacity Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA). The primers were designed with Primer3 software and listed in Supplementary Table 1

The 15 μL PCR reaction mix contained 3 μL of cDNA (10 ng/μL), 7.5 μL of iQ^{TM} SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA), 0.5 μL of primer mix (10 μM each), and 4 μL of ddH_2O. After 5 min denature at 95°C, 40 cycles will be performed: annealing and extension at 60°C for 45 seconds and denature at 95°C for 10 seconds. Dissociation curve was performed after finishing 40 cycles to verify the quality of primers and amplification. Relative expression of genes was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized to the house keeping gene β-actin or expressed as % of controls.

Western blot analysis

Approximately 80 mg of liver tissue was homogenized with RIPA lysis buffer containing 1 mM PMSF and freshly prepared proteinase inhibitors. The homogenates were centrifuged at 12,000 g at 4ºC for 10 min, and the protein concentration in the supernatants was determined by the BCA assay, and denatures at 90ºC for 10 min with Nupage loading buffer. Approximately 30 μg proteins were separated in the10% Nupage gel and transferred to the PVDF membrane. The membranes were blocked in 5% of the skim milk for 1 h at room temperature, followed by incubation with primary antibodies (CYP1A2 (1:500), CYP2B1 (1:500), CYP2E1 (1:500), CYP3A4 (1:500), CYP4 (1:500), CYP7A1 (1:500), and GAPDH (1:2000) at 4°C overnight. After washing the membranes with TBST 4 times, the secondary horseradish peroxidase (HRP) labelled anti-rabbit, or anti-mouse antibodies were added (1:5000) (Beyotime, Shanghai, China), and incubated at room temperature for 1 h, The enhanced chemiluminescent reagents (ECL) were used to detect the intensity of protein-antibody complexes, and intensity was semi-quantified with Quantity One software (Bio-Rad, USA).

Statistical analysis

Data were expressed as mean and standard error. The SPSS 19 software was used for statistical analysis. Data were analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test, and p value < 0.05 was considered significant.

Results

Animal general conditions

At the doses used in the present study, animals were healthy, without body weight loss and no mortality occurred. No significant elevations of serum ALT and AST were evident, and histology did not reveal overt lesions except for mild pathology in $HgCl_2$ and MeHg groups (data not shown). These results were confirmatory to our recent publications [11, 24, 27].

AhR and CYP1A expression

Aryl hydrocarbon receptor (AhR) mainly mediates the expression of CYP1A1 and CYP1A2 proteins. 2,3,7,8-Tetrachlorodibenzodioxin(TCDD) is a typical CYP1A inducer. When TCDD is combined with AhR, AhR is dissociated from the complex and transferred to the nucleus. It forms heteromeric dimers with AhR nuclear transport protein, and then induces the expression of target genes. In this way, TCDD and other AhR activators significantly induce the expression of CYP1A enzyme genes metabolism [17]. The present results showed that compared with the normal group, there was no significant change in the expression of *AhR*, *Cyp1a2* in 70W and Zuotai groups. The mRNA expression of *AhR* and *Cyp1a2* increased in $HgCl_2$ and MeHg groups, and the expression of *Cyp1a2* in $HgCl_2$ group was significant. The protein expression of CYP1A2 was also increased by $HgCl_2$ and MeHg (Fig. 1).

CAR and CYP2B expression

Constitutive androstane receptor (CAR) is a nuclear receptor of steroid hormones. It regulates the metabolizing enzymes and transporters in liver and small intestine. CAR mediates endogenous hormone or exogenous drug reactions, such as phenobarbital (PB), and transcriptionallyregulatesCYP2 enzymes metabolism [17]. The present results showed that compared with the normal group; there was no significant difference in the expression of *CAR*, *Cyp2b10* in 70W and Zuotai groups. The expression of *CAR*, *Cyp2b10* mRNA and CYP2B1 protein was increased by $HgCl_2$, and MeHg tended to increase *CAR* and *Cyp2b10* mRNA, but increased CYP2B1 protein expression (Fig.2).

CYP2E1 gene and protein expression

CAR mediates endogenous hormone or exogenous drug reactions, such as phenobarbital (PB), and transcriptionallyregulatesCYP2 enzymes. The present results showed that compared with the normal group; there was no significant difference in the expression of *Cyp2e1* mRNA and CYP2E1 protein in 70W and Zuotai groups. The expression of CYP2E1 protein was increased by $HgCl_2$ (Fig.3).

PXR and CYP3A expression

Pregnane X receptor (PXR) is a highly conserved ligand-dependent transcription factor, also known as a pregnant receptor. It is mainly expressed in the liver and partly expressed in the colon and small intestine. It plays an important role in the process of mammalian tumor formation, development, reproduction, carbohydrate metabolism, tissue growth, exogenous substance clearance and cardiovascular function. The regulation of the expression of Cyp3a11 by PXR mediated signaling pathway is an important pathway to influence drug metabolism [17].The results show that compared with the Control, there was no significant difference in the expression of *Cyp3a11* in each group. The expression of PXR was slightly increased in groups of $HgCl_2$, MeHg, and Zuotai, and there was no significant difference in the expression of PXR and *Cyp3a11*in 70W group (Fig.4). All treatments had no appreciable effects on CYP3A protein expression.

PPARα and CYP4 gene expression

Peroxisome proliferator-activated receptors (PPARs) nuclear receptor family regulates the expression of genes that control fatty acid synthesis, storage, and catabolism. PPARs mainly include PPARα, PPARβ and PPARγ. The activation of PPAR can improve insulin resistance, slow down atherosclerosis, and promote the metabolism of cholesterol in macrophages. PPARα regulates induction of CYP4A metabolism [17]. The present results showed that compared with the normal group, there was no significant difference in the expression of PPARα, *Cyp4a10* in 70W and the Zuotai groups. The expression of PPARα, *Cyp4a10* was slightly increased in groups of $HgCl_2$ and MeHg, but was not significant (Fig.5). All treatments had no effects on CYP4A protein expression.

FXR and CYP7A expression

Bile acid (BA) is the endogenous ligand of farnesoid X receptor (FXR), so FXR is also called the BA receptor. FXR is not only an important factor in regulating glucose and lipid metabolism, but also as a signal molecule involved in insulin signal transduction, oxidative stress, inflammation, hepatic stellate cell activation. Cholesterol 7α hydroxylase (Cyp7a1) is important for BA synthesis. When BA overloads in the liver, toxicity to liver cells occurs, including oxidative stress, inflammation, necrosis and even cirrhosis of the liver [28]. The results show that compared with the normal group, there was no significant difference in the expression of FXR, *Cyp7a1* in 70W and the Zuotai group. The expression of FXR was slightly increased in groups of $HgCl_2$ and MeHg, but was not significant. The expression of *Cyp7a1* in $HgCl_2$ group was significantly increased. $HgCl_2$ and MeHg increased CYP7A1 protein expression (Fig.6).

Discussion

Minerals (metals) in traditional medicines are the matter of debate on their efficacy and toxicity potentials [7, 20]. In the present research, we examined the effects of β-HgS-containing Zuotai and Zuotai-containing 70W on hepatic CYP 1-4 and CYP-7 families, and their corresponding nuclear receptors, compared to $HgCl_2$ and MeHg at both mRNA and protein levels. Briefly, 70W at 1 to 5-fold clinical doses and Zuotai (30 mg/kg, po) for 7 days did not produce significant effects on the liver CYP450 gene and protein expressions; while $HgCl_2$ at equivalent Hg dose increased the expression of CYP1A, CYP2B, CYP2E1, and CYP7A at the transcription and/or protein levels, MeHg at 1/10 Hg dose produced similar effects, but to a lesser extent. These results further demonstrated that chemical forms of metals are a major determinant of their biological effects [24-27, 29-31].

Zuotai in Tibetan Medicines

The Tibetan medicine has thousand years of history and is still used in the world today for a variety of diseases, including liver diseases [1-6]. Herbal-metallic preparations are believed to assist the delivery of drugs to the target, contribute to therapeutic effects, and reduce toxicity [7, 20]. 70W is one of famous Tibetan medicines listed in the 2015 Edition of Chinese Pharmacopoeia in the treatment of various diseases [3-7]. We have shown that 70W is effective against CCl_4-induced liver injury [11] and protected LPS plus MPTP-induced neurotoxicity in mice [8], and the present study further demonstrated that the hepatoprotective effects of 70W is not due to the inhibition of CYP450 to reduce CCl_4 bioactivation, rather than due to activation of the Nrf2 antioxidant pathway [11].

Zuotai is included in 70W in a small amount [2-4, 8]. The chemical speciation, spatial distribution of mercury from Zuotai are different from that of $HgCl_2$ [7, 18], resulting in differential toxicity when compared to environmental mercury compounds ($HgCl_2$ and MeHg) in experimental animals and cultured human cells [24-27, 29-31]. In patients taking Zuotai-containing Tibetan medicines, mercury toxicity to the liver and kidney was mild, tolerable and reversible [10, 21-23]. The present study demonstrated that Zuotai-containing 70W at clinical doses had minimal effects on hepatic CYP450, supporting the notion that Zuotai and 70W at clinical doses are safe [19, 21-23].

Mercury effects on CYP-1 family

Cytochrome P450 1A1 (CYP1A1) is a hepatic and extrahepatic enzyme that is regulated by the AhR signaling pathway and is regarded as carcinogen activation CYP450 family [32]. CYP-1 family includes CYP1A1, CYP1A2, and CYP1B1 and CYP1A1/CYP1A2 has become a therapeutic tool for the bioactivation of prodrugs, particularly cytotoxic agents. Little is known about effects of 70W on CYP1A family. We have shown previously that oral Zuotai (β-HgS) and cinnabar (α-HgS) had minimal effects of hepatic P4501A family gene expression [33, 34]. However, in rats, Zuotai at higher doses decreased CYP1A2 activity [35]. In comparison, the effects of $HgCl_2$ on CYP1A expression were more dramatic. In Zebrafish, low dose (0.1 LC50) of $HgCl_2$ increased CYP1A1, but at higher doses (0.4 and 0.8 LC50), the expression of CYP1A1 was suppressed [36]. Zuotai at the concentration of 0.2 mg/mL (below LC50) could increase CYP1A1 and CYP1B1 in Zebrafish [36]. In the mouse heart, kidney and lung, $HgCl_2$ (2.5 mg/kg, ip) increased CYP1A1, along with other CYP450 isoforms [37]. However, in the interactions with AhR ligand TCDD, TCDD induction of *Cyp1a1*, *Cyp1a2*, and *Cyp1b1* was suppressed by $HgCl_2$ (2.5 mg/kg, ip) in livers of mice [38]. In the present study, $HgCl_2$ at 33.6 mg/kg increased CYP1A2 at mRNA and protein levels and tended to increase AhR mRNA, largely in agreement with the above literature. In another study, mice chronically (6 weeks) received $HgCl_2$ (32 mg/kg) and MeHg (2.6 mg/kg), expression of hepatic Cyp1a1 and Cyp1b1 was increased by MeHg, and tended to increase by $HgCl_2$, while cinnabar (HgS, 300 mg/kg) and cinnabar-containing An-Gong-Niu-Huang Wan were ineffective [39]. Thus, Effects of mercury on CYP1 family are dependent on the mercury forms, the dose, route, and duration of administration.

Mercury effects on CYP-2 and CYP-3 family

CYP-2 family is easily induced by many xenobiotics such as phenobarbital. The CAR is shown to play a crucial role in the activation of CYP2B genes by xenobiotics [17]. CYP-2 family mainly includes CYP2B subfamily and CYP2E1. CYP2E1 metabolizes an extensive array of pollutants, drugs, and other small molecules, often resulting in bioactivation to reactive metabolites, which in turn damage mitochondria [40]. $HgCl_2$-induced hepatotoxicity and oxidative stress is partially mediated through its effects on CYP2E1 [41]. $HgCl_2$ (2.5 mg/kg, ip) increased *Cyp2b9*, *Cyp2b10* in mouse heart [38], and $HgCl_2$ (33.6 mg/kg, po) increased *Cyp2b10* expression in the livers of mice [34]. Similar to CYP1A2, higher doses of systemic $HgCl_2$ decreased hepatic Cyp2e1 in livers of rats [35]. Under the present experimental conditions, *Cyp2b10* mRNA and CYP2B protein expression were increased by $HgCl_2$ and MeHg only.

CYP3A is the most abundant subfamily of CYP450, with the highest content in the liver and intestines, and is involved in the metabolism of clinical drugs [14, 15].CYP3A can be induced or inhibited by a variety of substances, influence factors and individual differences. This enzyme is an activator of aflatoxin B, can be transformed into liver cancer cells induced by carcinogens. CYP3A can catalyze the structurally different substrates, including calcium channel blockers and antiarrhythmic drugs. In the present study conditions, 70W and mercury compounds had minimal effects on *Cyp3a11* mRNA and CYP3A protein

expression. However, the polymorphisms in human CYP3A genes (CYP3A4. CYP3A5 and CYP3A7) may modify the response to dietary MeHg exposure during early life development [42]. In mice chronically dosed with $HgCl_2$ (32 mg/kg) and MeHg (2.6 mg/kg), cinnabar (HgS, 300 mg/kg) and cinnabar-containing An-Gong-Niu-Huang Wan, expression of hepatic Cyp3a11 and 3a25 were increased [39]. The length of Hg compound administration makes a difference as compared to the present study.

Mercury effects on CYP-4 and CYP-7 family

CYP4A is involved in lipid metabolism and is regulated by PPARα, their dysregulations are implicated in xenobiotics induced adverse effects leading to various human diseases [17]. Researchers found mercury exposure is associated with increased risk of cardiovascular disease and profound cardiotoxicity, and their results show that mercury treatment caused a significant induction of the cardiac hypertrophy markers, alone with CYP4A genes (*Cyp4a10, Cyp4a12, Cyp4a14*) [37]. In the present study, 70W and Zuotai at 1-5 fold clinical doses do not have appreciable effects on PPARα and *Cyp4a10*. $HgCl_2$ produced a slight increase in PPARα and *Cyp4a10*in previous studies [33], and in mice chronically dosed with $HgCl_2$ (32 mg/kg) and MeHg (2.6 mg/kg), Expression of *Cyp4a10* was increased, but cinnabar (HgS, 300 mg/kg) and cinnabar-containing An-Gong-Niu-Huang Wan was ineffective [39]. However, in the present study, only the trends of increase were evident.

CYP7A1 is a rate-limiting enzyme for bile acid synthesis and is regulated by FXR [28]. Little is known on the effects of mercury compounds on FXR and CYP7A1 expression. This is the first reports to show that $HgCl_2$ and MeHg had capability of induction of *Cyp7a1* mRNA and CYP7A1 protein. The biological effects of CYP7A1 induction by $HgCl_2$ and MeHg warrant further investigation.

Conclusions

The present study showed β-HgS and β-HgS containing 70W (1-5-fold of clinical dose) did not produce appreciable effects on hepatic CYP450 enzyme gene/protein expression compared to equal Hg content as $HgCl_2$ or 1/10 of Hg content as MeHg, suggesting that (1) the protection of 70W against CCl_4 hepatotoxicity is not due to inhibition of CYP450 (CYP2E1); (2) 70W appeared to be safe under recommended clinical doses; and (3) $HgCl_2$ and MeHg had significant on CYP450 expression, their potential interactions with drugs should be alerted.

Abbreviations

70 Wei-Zhen-Zhu-Wan (70W, also called Rannasangpei; 70 Flavors Pearl Pill); Cytochrome P450 (CYP450); Aryl hydrocarbon receptor (AhR); Constitutive androstane receptor (CAR); Pregnane X receptor (PXR); Peroxisome proliferator-activated receptors (PPARs); farnesoid X receptor (FXR).

Declarations

Acknowledgements

Not applicable.

Authors' contributions

All authors involved in writing the paper. YN, SFX, YLL and XRZ performed the experiments. YN, SFX, and YLL analyzed and interpreted the data. YN, SFX, JL, LXW conceived and designed the experiments. CL and LXW contributed reagents and materials. YN, SFX, JL, CL and LXW wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval

All animal care and experimental protocols are complied with the Animal Management Guidelines of the Chinese Ministry of Health and approved by Animal Use and Care Committee of Zunyi Medical University (2013-05).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Supplementary Table 1

Supplementary Table 1 not provided with this version of the manuscript.

Figures

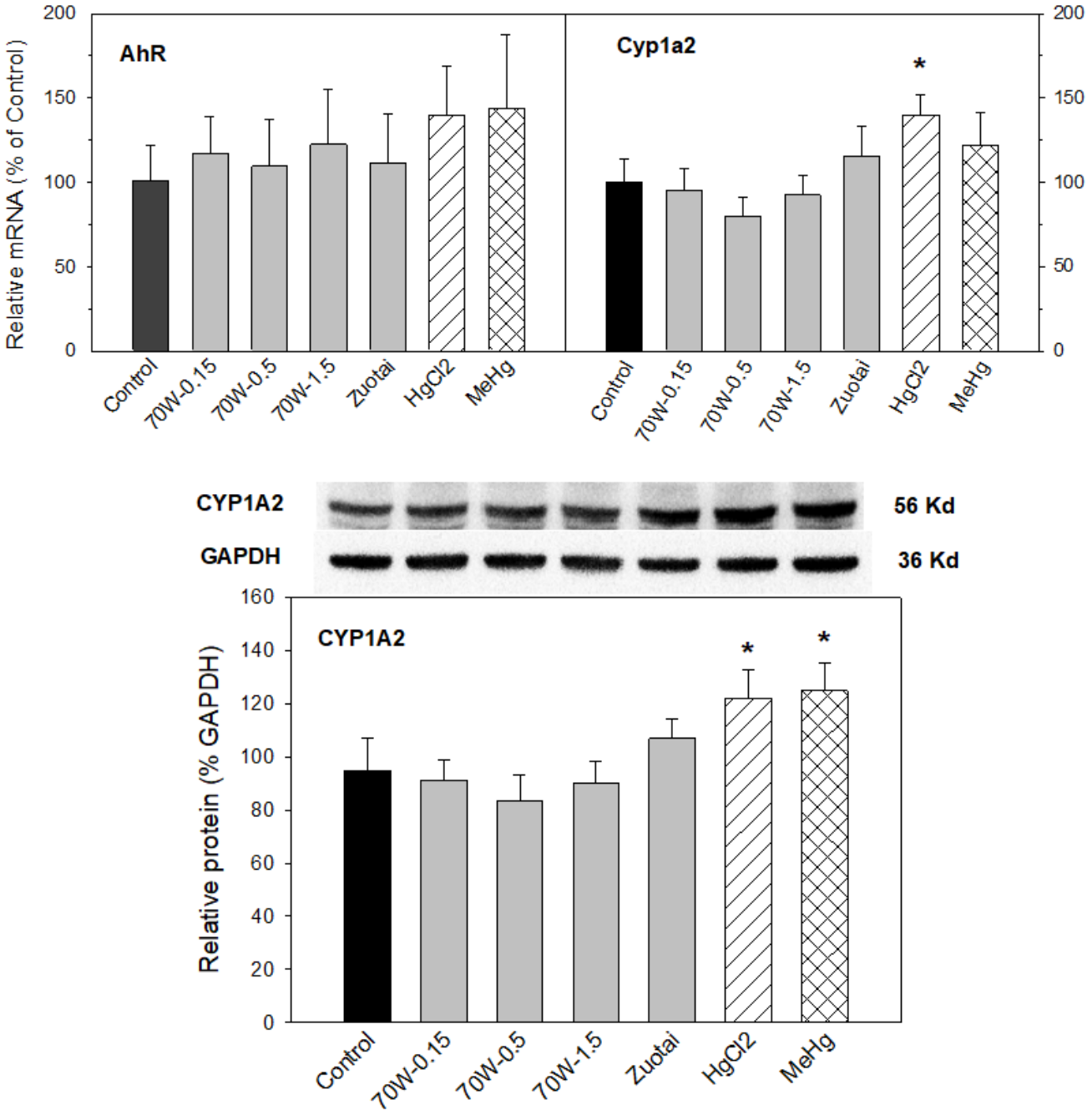

Figure 1

Effect of 70W and mercury compounds on AhR and CYP1A gene and protein expression. Mice were given 70W 0.15, 0.5, and 1.5 g/kg, po. Zuotai (30 mg/kg, po), HgCl2 (33.6 mg/kg, po), and MeHg (3.1 mg/kg, po) daily for 7 days, and hepatic RNA and protein were extracted for RT-PCR and Western-blot analysis, respectively. Data are mean ± SE, n = 5. *Significantly different from control, p< 0.05.

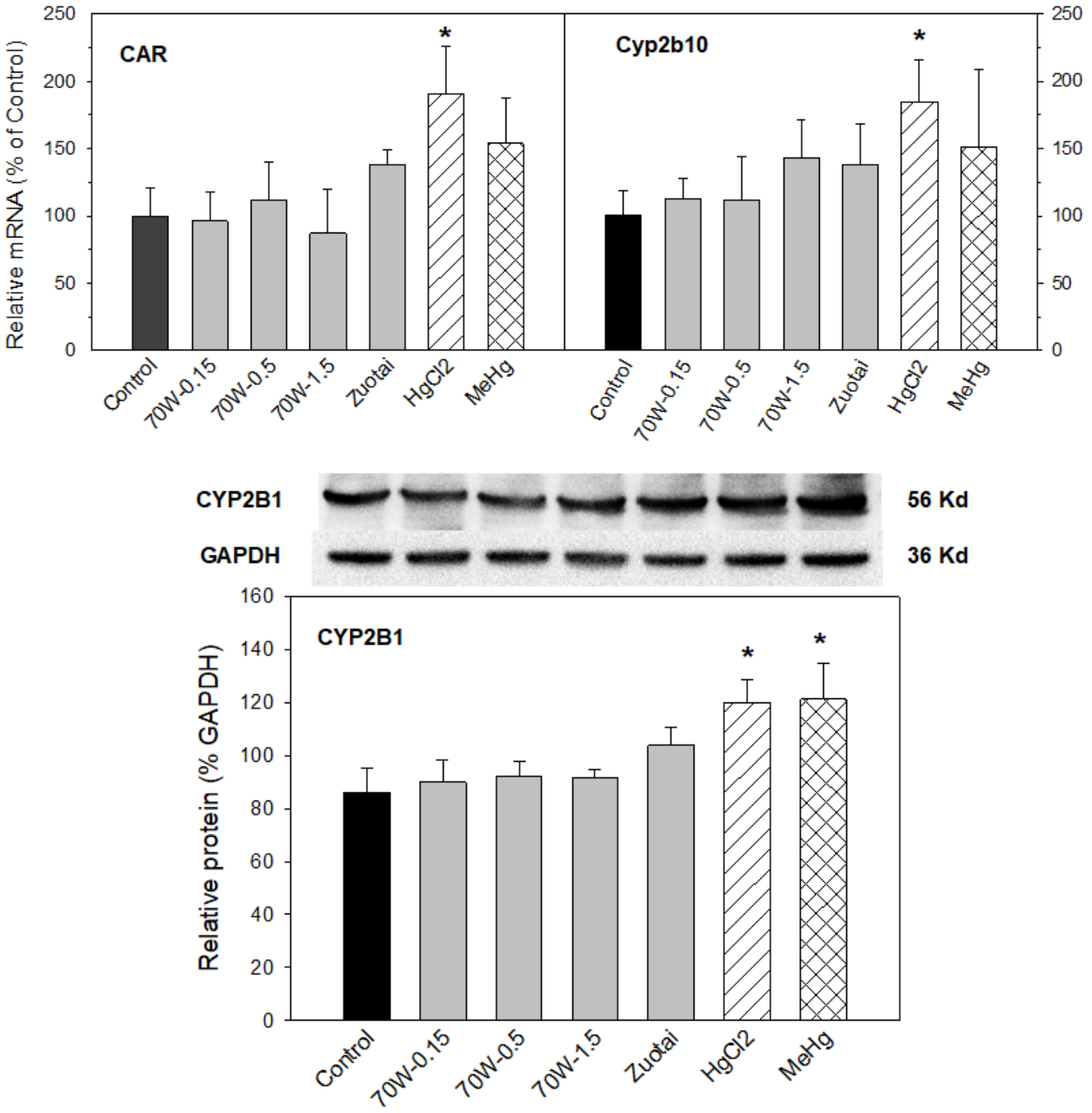

Figure 2

Effect of 70W and mercury compounds on CAR and CYP2B expression. Experiments were detailed in Figure 1. Data are mean ± SE, n = 5. *Significantly different from control, p< 0.05.

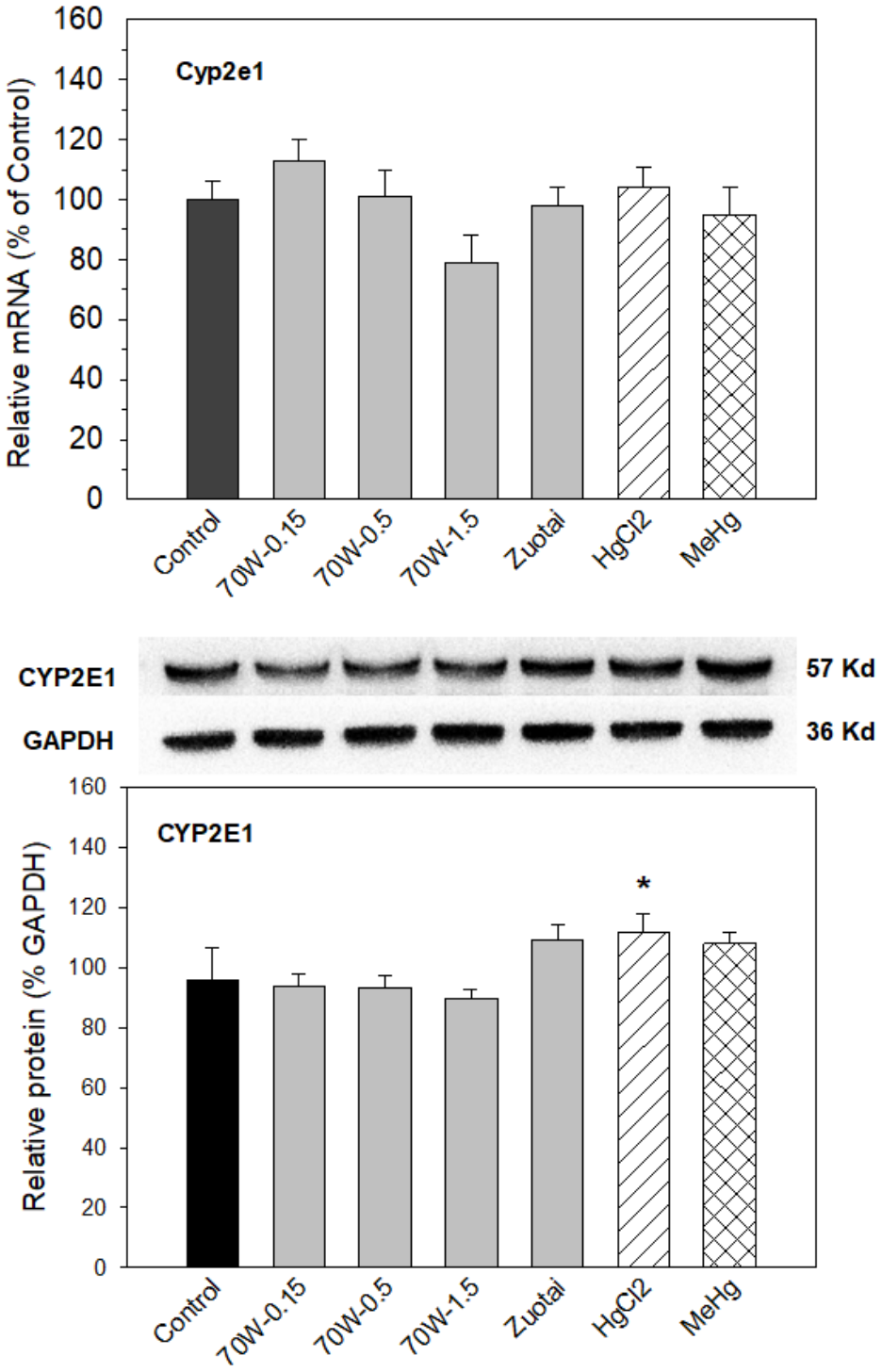

Figure 3

Effect of 70W and mercury compounds on CYP2E1 expression. Experiments were detailed in Figure 1. Data are mean ± SE, n = 5. *Significantly different from control, $p < 0.05$.

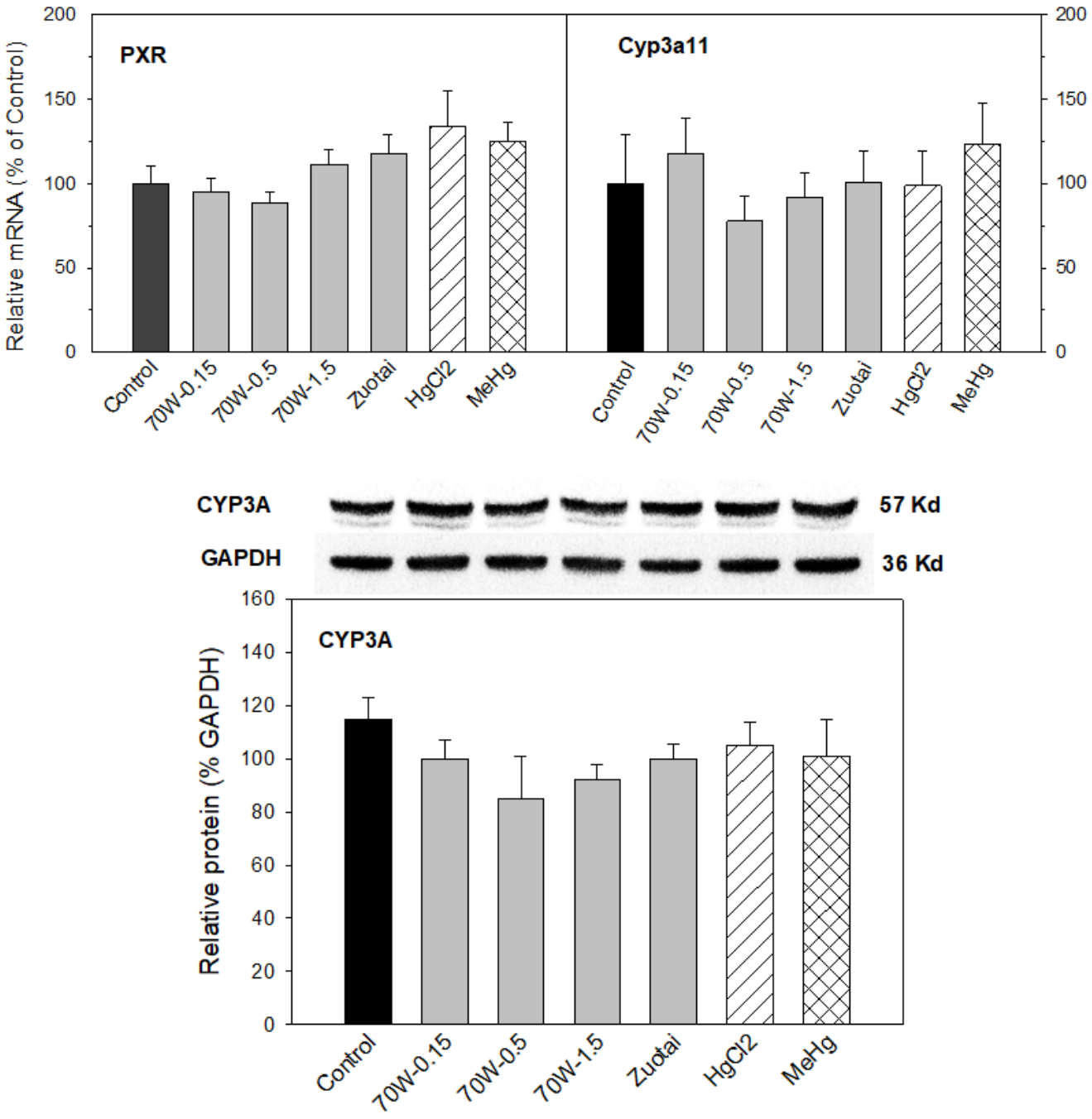

Figure 4

Effect of 70W and mercury compounds on PXR and CYP3A expression. Experiments were detailed in Figure 1. Data are mean ± SE, n = 5. *Significantly different from control, p< 0.05.

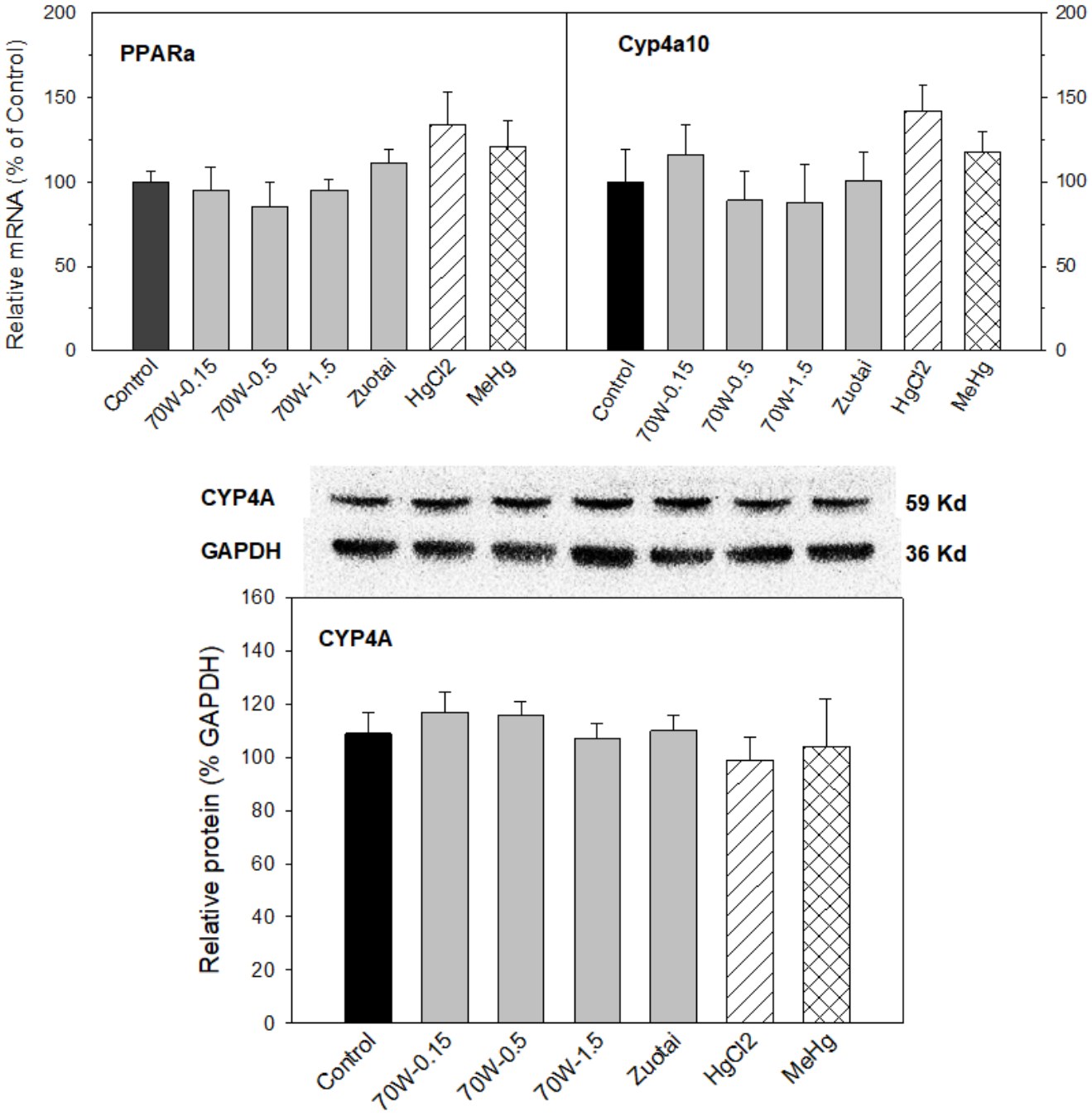

Figure 5

Effect of 70W and mercury compounds on PPARα and CYP4 expression. Experiments were detailed in Figure 1. Data are mean ± SE, n = 5. *Significantly different from control, p< 0.05.

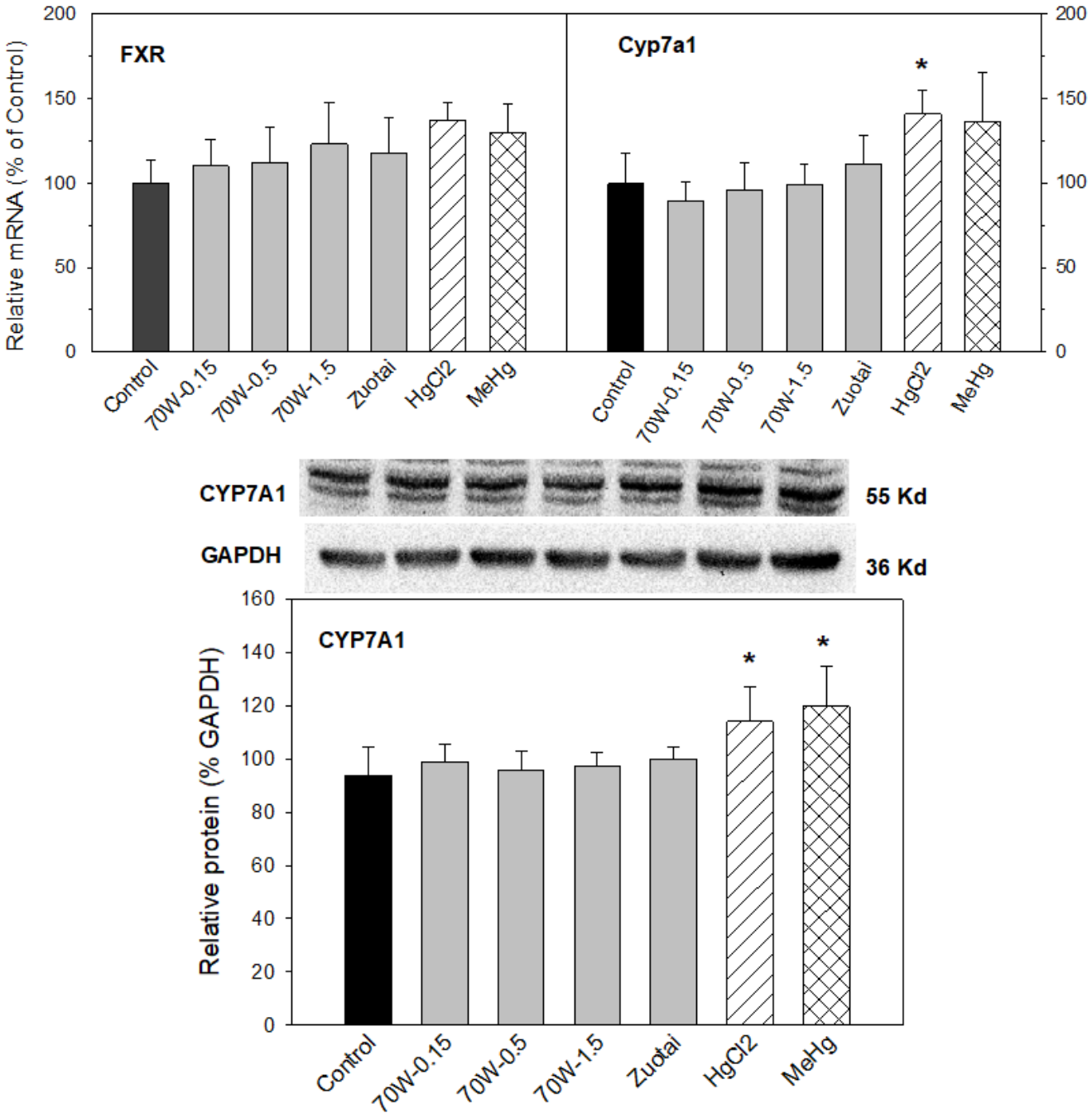

Figure 6

Effect of 70W and mercury compounds on FXR and Cyp7a1 gene expression. Mice were given 70W 0.15, 0.5, and 1.5 g/kg, po, Zuotai (30 mg/kg, po), HgCl2 (33.6 mg/kg, po), and MeHg (3.1 mg/kg, po) daily for 7 days, and hepatic total RNA was extracted for RT-PCR analysis. Data are mean ± SE, n = 5.
*Significantly different from control, p< 0.05.