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Agonist of farnesoid X receptor protects against bile acid induced damage and oxidative stress in mouse placenta – A study on maternal cholestasis model

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ABSTRACT

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific disorder, which is characterized by raised serum bile acid level and potential adverse fetal outcome. Farnesoid X receptor (FXR), also known as a bile acid receptor, was found to be expressed in placenta with low level. Whether activation of FXR by specific agonists could regulate the pathogenesis of ICP is still unclear.
Methods: A model of maternal cholestasis was induced by administration of 17α-ethynylestradiol (E2) in pregnant mice for 6 days. We explored the regulatory effect of WAY-362450 (W450), a highly selective and potent FXR agonist on placenta.
Results: In this study, we demonstrated that administration of E2 increased bile acid levels in mouse serum, liver and amniotic fluid. Bile acid levels were significantly decreased after W450 treatment. W450 protected against the impairment of placentas induced by E2, including severe intracellular edema and apoptosis of trophoblasts. Moreover, W450 significantly induced the expressions of FXR target bile acid transport gene ATP-binding cassette, sub-family B (MDR/TAP), member 11 (*Abcb11*;*Bsep*) in placenta. W450 could also attenuate placental oxidative stress and increase the expressions of antioxidant enzymes *Prdx1* and *Prdx3*.
Discussion and conclusion: In conclusion, our data demonstrated that FXR agonist W450 modulated bile acid balance and protected against placental oxidative stress. Thus, our results support that potent FXR agonists might represent promising drugs for the treatment of ICP.

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific disorder, which predominantly occurs in late pregnancy. ICP is characterized by maternal pruritus, raised serum bile acid level and liver transaminases [1]. Although ICP causes little maternal risk, the disease is associated with an increased risk of adverse perinatal outcomes, such as fetal distress, spontaneous preterm delivery, and sudden intrauterine death [1–3]. The etiology of ICP is complicated and not fully understood, it was suggested that environmental, genetic and reproductive hormonal factors might contribute to its pathogenesis [4–6].

High estrogen concentration in the third trimester of pregnancy has been reported to be one of the major contributors to the pathogenesis of ICP [4,7]. Even in some non-pregnant patients, taking oral contraceptives containing 17α-ethynylestradiol could lead to cholestasis [8]. Farnesoid X receptor (FXR, NR1H4) is a bile acid sensor and balancer [9]. It was indicated that high estrogens or its metabolites might inhibit FXR activity through activating estrogen receptor (ER), leading to dysregulated bile acid homeostasis in late pregnant mice [10]. Numerous reports have suggested high

Abbreviations: Abcb11, ATP-binding cassette, sub-family B (MDR/TAP), member 11; Abcc2, ATP-binding cassette, sub-family C (CFTR/MRP), member 2; Abcb4, ATP-binding cassette, sub-family B (MDR/TAP), member 4; Abcb1a, ATP-binding cassette, sub-family B (MDR/TAP), member 1A; Slco1a1, solute carrier organic anion transporter family, member 1a1; Atp8b1, ATPase, class I, type 8B, member 1; Cat, Catalase, Sod1, superoxide dismutase 1; Sod2, superoxide dismutase 2; Gstm3, glutathione S-transferase mu 3; Gsta4, glutathione S-transferase alpha 4; Nox4, NADPH oxidase 4; Gpx7, glutathione peroxidase 7; Fmo2, flavin containing monooxygenase 2; Prdx1, peroxiredoxin 1; Prdx3, peroxiredoxin 3.

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concentration of bile acids in circulation could increase the risk of deleterious consequence of fetus in ICP [11–13]. The pathogenesis of ICP may also be related to abnormal biliary transport during late pregnancy [4,14]. In mammals, the synthesis and metabolism of bile acids is tightly regulated by members of the nuclear hormone receptor superfamily including FXR [9]. FXR plays a central role in maintaining bile acid homeostasis by targeting genes involved in bile acid synthesis, secretion and enterohepatic circulation. Ligand activated-FXR promotes bile acid export from liver through inducing the expressions of bile salt export pump (BSEP, ABCB11) and multidrug resistance protein 3 (MDR3, ABCB4; *Mdr2* in mouse) whereas it down-regulates bile acid import via repressing the expression of sodium/taurocholate co-transporting polypeptide (NTCP, SLC10A1) [15]. Additionally, activated FXR also attenuates *de novo* bile acid synthesis through induction of small heterodimer partner (SHP, NR0B2), which transcriptionally represses the expression of cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1) [16–18].

Whether the administration of FXR agonist affects placental function is still unclear. However, the role of FXR in placenta during the course of ICP has never been studied. In the present study, we addressed this issue by studying the effect of a specific agonist of FXR, WAY-362450 (W450) in a mouse model of maternal cholestasis.

1. Materials and methods

1.1. Animal experiment

Adult female C57BL/6 mice (10-week old) used throughout the study were from Shanghai Laboratory Animal Center (Chinese Academy of Sciences, Shanghai, China). The animals were maintained on standard chow and water *ad libitum*. The animals received humane care. All studies were approved by the institutional ethical review board of the International Peace Maternity and Child Health Hospital (IPMCH), Shanghai Jiao Tong University (SJTU).

Estrogen induced maternal cholestasis was applied as previously reported [19], with some modification. On day 14 of pregnancy, mice were randomly divided into three experimental groups (n = 6–8/group). In the control group, mice were treated with vehicle. In the E2 group, mice were injected with 17α-ethynylestradiol (E2, Sigma–Aldrich Inc., St. Louis, MO; 5 mg/kg, *s.c.*) and gavaged with vehicle. In the E2-W450 group, mice were injected with E2 (5 mg/kg, *s.c.*) and gavaged with 30 mg/kg W450 (Selleck Chemicals, Houston, TX). The mice were treated once daily for 6 days.

To further testify the effect of high estrogen level induced bile acid accumulation on animals, we also carried out another maternal cholestasis model by direct bile acid (here we used cholic acid, CA) feeding as previously reported [20]. Briefly, female mice (10–12-week-old) were randomly divided into three experimental groups. Mice were fed with a control diet or a diet supplemented with 0.5% CA (Sigma-aldrich) for 1 week before mating and throughout gestation. From day 13 of pregnancy, in the control group and the CA group, mice were treated with vehicle; in the CA-W450 group, mice were gavaged with 30 mg/kg W450 once daily for 7 days (n = 6–8/group). At the end of the study, all animals were anesthetized with sodium pentobarbital (75 mg/kg, IP) and sacrificed by exsanguinations. Blood was gathered from the *vena cava* and serum was separated. Placenta and liver tissues were also collected. Tissue samples were immersed 10% neutral buffered formalin for histological examination, or immediately frozen in liquid nitrogen and kept at −80 °C until further analysis.

1.2. Clinical samples

Frozen tissues of human term placentas (20 from normal pregnancy and 11 from ICP) were retrieved from the archives of Department of Bio-bank, IPMCH, SJTU. Ethical approval for the use of tissues in this study has been obtained from institutional ethical review board of IPMCH, SJTU.

1.3. Cell culture

Human hepatoma cell line (Hep3B) and human choriocarcinoma cell lines (JAR and JEG-3) were obtained from Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and 1% penicillin/streptomycin in a Thermo Scientific incubator (37 °C, 5% CO_2).

1.4. Biochemical measurements

Mouse liver tissues were homogenized in 75% ethanol and hepatic bile acids were extracted at 60 °C for 2 h. Hepatic and serum bile acids were measured using a kit from DiaSys Diagnostic Systems (Holzheim, Germany) as described previously [21]. Placental lipid peroxidation was determined by detecting malondialdehyde (MDA) content as described [21]. MDA was extracted and measured using a commercial kit (Genmed, Shanghai, China) according to the manufacture's protocol.

1.5. Histopathological examination and TUNEL assay

The mouse placentas were fixed with formalin and embedded in paraffin. Tissue blocks were cut for 5-μm thick sections, which were subjected to hematoxylin and eosin staining (H&E) or immunohistochemistry for detecting ABCB11 expression (Santa Cruz Biotech, Santa Cruz, CA).

To carry out *in situ* cell apoptosis detection, placental sections were deparafinized with xylene, rehydrated in graded ethanol solutions. Then, DNA nicking was detected by the terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick end labeling (TUNEL) assay, according to the manufacturer's instruction (Roche Applied Science, Mannheim, Germany). Positive staining was indicated by a green fluorescence signal. Cell nucleus was counterstained with propidium iodide (blue signal, Beyotime, Nanjing, China).

1.6. Total RNA isolation and quantitative RT-PCR (qRT-PCR) assay

Mouse or human placental tissues were homogenized in 1 ml Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA was extracted and treated with AccuRT gDNA Removal Kit (abmgood, Richmond BC, Canada) to eliminate genomic DNA contamination. Subsequently, cDNA was synthesized using EasyScript Reverse Transcriptase (abmgood, Richmond BC, Canada) according to the manufacturer's protocol. qRT-PCR using a Kapa SYBR Fast qPCR Kit (Kapa Biosystems, Woburn, MA) was performed to detect mRNA expression on a StepOnePlus quantitative PCR system (Applied Biosystems, Foster City, CA) according to the manufacturer's instruction. The primers for qRT-PCR are listed in Supplementary table 1. Relative mRNA level of target gene was calculated using the $2^{-\Delta\Delta Ct}$ method for the difference between the CT value of for the target gene and the mean CT value for the endogenous control (18s rRNA) in the same sample, and was expressed as a fold change between control and treated group.

1.7. Western blot assay

Tissues were homogenized in a RIPA buffer (150 mM NaCl, 1% NP- 40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris–HCl, 1 mM aprotinin, 1 mM PMSF and 10 μg/ml

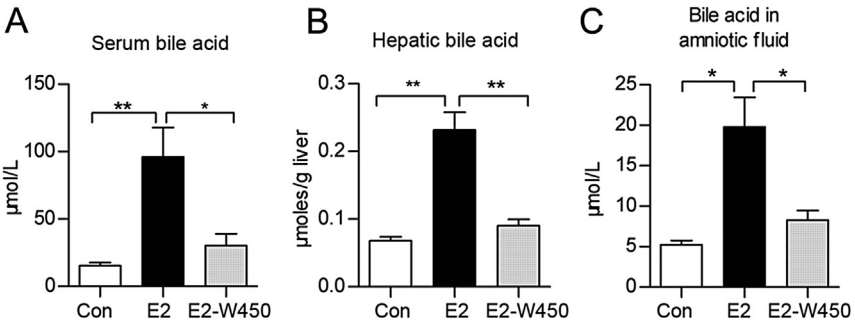

Fig. 1. Administration of W450 lowered circulatory bile acid level in mouse model of maternal cholestasis. Pregnant C57BL/6 mice were injected with 17α-ethynylestradiol (E2 group) or simultaneously gavaged with WAY-362450 (E2-W450 group) once daily for 6 days, vehicle treated mice were used as Control (Con) group. The bile acid content in maternal serum (A), liver (B) and in amniotic fluid (C) was determined in the three groups. (n = 6–8 in each group, $^{*}p < 0.05$. $^{**}p < 0.01$,Student's t-test).

leupeptin) and the cells were suspended in an SDS lysis buffer (40 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% SDS, 1 mM aprotinin, 1 mM PMSF and 10 μg/ml leupeptin). Western blot assays were performed as previously described [21]. In brief, lysates were separated by SDS-PAGE electrophoresis in 10% gels. Total proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The membrane was then incubated with antibodies against FXR (1:500, Santa Cruz Biotech, Santa Cruz, CA) or β-Actin (1:2000, Biomart, Shanghai, China) and corresponding secondary antibodies (Jackson ImmunoResearch, West Grove, PA), and subsequently developed using the enhanced chemiluminescent (ECL) assay kit (Tiangen Biotech, Beijing, China).

1.8. Statistics

Data are shown as means ± S.E.M. Statistical analysis was performed using the GraphPad Prism 5 software (GraphPad Software, San Diego, CA) by two-tailed Student's t test, Fisher's exact test or one-way ANOVA. A value of $p < 0.05$ was considered statistically significant.

2. Results

2.1. Administration of W450 lowered circulatory bile acid level in mouse model of maternal cholestasis

Administration of E2 for 6 days caused significantly elevated bile acid levels in serum, liver and amniotic fluid of pregnant mice (Fig. 1A-C, $p < 0.05$, $p < 0.01$ versus Con group), whereas co-administration of W450 significantly attenuated the increment of

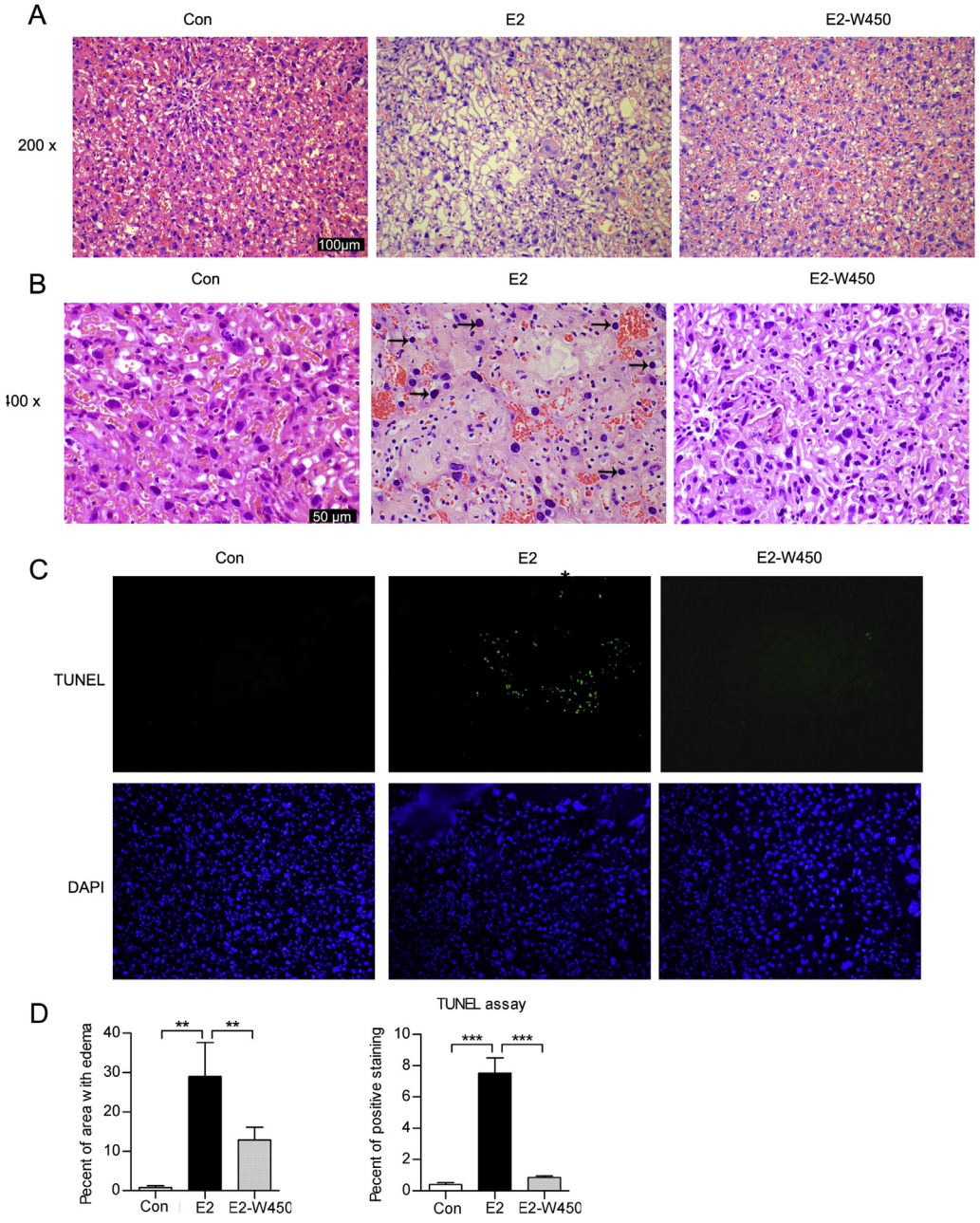

Fig. 2. Administration of W450 attenuated intracellular edema and cell apoptosis in mouse placenta. (A) Mouse placental sections from the Con, E2 and E2-W450 groups were stained with H&E. Representative images were shown (original magnification, × 200) (upper panel) with local magnifications shown below. (B) Trophoblastic cells undergoing apoptosis (black arrow) and edema area (star) were shown in representative images (original magnification, × 400). (C) By TUNEL, apoptosis in mouse placentas from the three groups was detected. Green immunofluorescence staining indicates a positive signal and cell nucleus was counterstained with propidium iodide (PI, blue signal). Representative images were shown (original magnification, 200 ×). (D) Left and right bar chart showed the percent of area undergoing edema (A) and the percent of TUNEL positive nuclei (C), respectively, as calculated with LAS image analysis software (Leica Microsystems Heidelberg GmbH, Germany), (n = 6–8 in each group, $^{**}p < 0.01$, $^{***}p < 0.001$, Student's t-test).

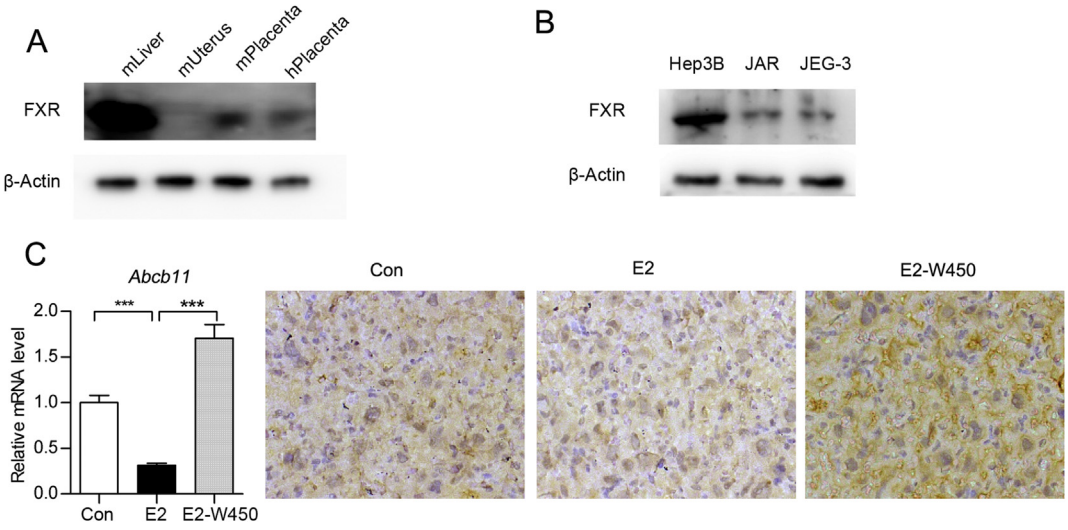

Fig. 3. Activation of FXR by W450 regulated expression of the bile acid export gene *Abcb11* in mouse placenta (A) FXR expressions in mouse liver (mLiver), mouse uterus (mUterus), mouse placenta (mPlacenta) and human placenta (hPlacenta) were detected by Western blot. (B) FXR expressions in human hepatoma cell line (Hep3B) and choriocarcinoma cell lines (JAR and JEG-3) were detected by Western blot. (C) Mouse placental tissues from the Con, E2 and E2-W450 group were subjected to qRT-PCR assay for determination of ATP-binding cassette, sub-family B (MDR/TAP), member 11 (*Abcb11*;*Bsep*) mRNA levels (left bar chart)as well as immunohistochemical assay of ABCB11 protein level (right images). (n = 6–8 in each group, $^{***}p < 0.001$, Student's t-test).

bile acid (Fig. 1 A–C, $p < 0.05$, $p < 0.01$ versus E2 group). Thus, selective agonist of FXR W450 is effective in lowering circulatory bile acid in mouse model of maternal cholestasis.

2.2. Administration of W450 attenuated intracellular edema and cell apoptosis in mouse placenta

By histopathological examination, we demonstrated that 6-day administration of E2 resulted in severe atrophy and intracellular edema of trophoblasts in mouse placenta, and apoptosis of trophoblasts in some fields, which are similar to the pathological features observed in rat in a previous report [22]. The placental histological damages by E2 were substantially ameliorated by W450 (Fig. 2A–B and D left panel). Similar effect of W450 was also observed in another mouse model of ICP (Supplementary Fig. 1), which was induced by cholic acid feeding as described in "Materials and methods".

To confirm the existence of apoptosis, the TUNEL assay was employed. The apoptosis signal was distributed all over the E2 placental sections, with dense distribution in some fields. However, apoptosis signal was rarely observed in placentas from E2-W450 group (Fig. 2C and D right panel). Thus, FXR agonist W450 is effective to protect against placental edema and cell apoptosis.

2.3. FXR was detected in trophoblastic cells of placenta

As previous reports by others [23,24], we consistently ascertained the existence of FXR in mouse and human placental tissues by western blot assay, although the protein levels were much lower than that in mouse liver (Fig. 3A). We further confirmed its expression in trophoblasts of choriocarcinoma cell lines JAR and JEG-3, again not as high as in liver cancer cell line Hep3B, which is used as a positive control (Fig. 3B).

2.4. Activation of FXR modulated expressions of the bile acid transporters in mouse placenta

To determine whether W450 could activate FXR and impact bile acid transport in placenta as such in liver, we examined the mRNA expressions of *Abcb11/Bsep*, *Abcb4/Mdr2*, *Abcb1a/Mdr3*, *Abcc2/Mrp2*, *Slco1a1/Oatp1* and *Atp8b1/FIC1* in mouse placentas from vehicle or W450 treated mice. We found that *Abcb11*, *Abcc2* and *Abcb4* were strongly induced by W450 ($p < 0.001$, $p < 0.01$ versus Vehicle group), whereas *Abcb1a* level was slightly decreased (Supplementary Fig. 3C). The expressions of *Slco1a1* and *Atp8b1* were not obviously changed.

More importantly, we further demonstrated the decreased mRNA expressions of *Abcb11* in E2 group were increased with significance in E2-W450 group (Fig. 3C, $p < 0.001$). The ABCB11 protein expression in mouse placenta displayed corresponding changes, *i.e.* weakened by E2 and intensified by W450 (Fig. 3C, IHC). Another FXR agonist GW4064 was employed to testify the result. The significantly raised mRNA expressions of *ABCB11* were observed in Hep3B, JAR and JEG-3 cells treated by GW4064 as well as by W450 when compared with vehicle treated cells (Supplementary Fig. 3). Thus, Activation of FXR by W450 is effective to activating bile acid transport related target genes in placenta, which may contribute to bile acid regulation in fetal-placental-maternal triad.

2.5. W450 attenuated oxidative stress and increased Prdx1/Prdx3 expression in placenta

Increased degree of lipid peroxidation (as determined by MDA assay, Fig. 4A) was observed in the placentas from E2 group, indicating enhanced oxidative damage. The elevated MDA level was largely blunted in the placentas from the E2-W450 treated group. Furthermore, a profile of oxidative response related genes were examine by qRT-PCR assay, including *Cat*, *Sod1*, *Sod2*, *Gstm3*, *Gsta4*, *Nox4*, *Gpx7*, *Fmo2*, *Prdx1*, and *Prdx3* as shown in Fig. 4B and supplementary Fig. 4. Among which, the mRNA expression incrementations of *Fmo2*, *Prdx1 and Prdx3* induced by W450 reached the significance ($p < 0.05$, $p < 0.01$ versus E2 group). Taken together, we showed that W450 attenuated placental oxidative stress and increased anti-oxidative genes expression in mouse placentas.

2.6. Elevated oxidative stress level with decreased PRDX1 and PRDX3 expressions in human placentas from ICP patients

To validate the *in vivo* results from mouse model, we also determined the lipid peroxidation level in human placenta. We observed an elevation of oxidative stress level ($p < 0.001$, Fig. 5A) in

the placentas from ICP patients. In addition, the mRNA expressions of *PRDX1* and *PRDX3* were decreased in ICP placentas ($p < 0.05$, $p < 0.01$, Fig. 5B). However, we could not observe the significantly changed FMO2 level between the Normal and ICP group ($p = 0.518$, supplementary Fig.5). These data suggested that lowered expression on anti-oxidative genes and enhanced oxidative stress level might contribute to the progression and adverse clinical outcomes of ICP in human.

3. Discussion

Raised serum bile acid is a major feature of ICP. According to previous studies, the risk of adverse pregnancy outcome was closely associated with high concentration of circulatory bile acids during the progression of ICP [11–13]. We demonstrated here that total bile acids in maternal serum, liver and amniotic fluid were significantly elevated in E2 induced cholestastic pregnancies, and were reduced by W450 treatment.

Toxic bile acid can disrupt the integrity of cell membranes by its detergent effect on lipid components. In rat hepatocytes, chenodeoxycholic acid was demonstrated to generate reactive oxygen species (ROS) and consequently cause hepatocyte apoptosis [28,29]. ROS could also oxidatively modify lipids on plasma and mitochondrial membrane and consequently cause cell apoptosis. In an obstructive cholestastic pregnant rat model, investigators observed that accumulated hydrophobic bile acids in the placental and fetal compartment, leading to significant oxidative damage and apoptosis in placenta and fetal liver [29,30]. Under physiological condition, the placenta plays an essential role in protecting the fetus from toxic bile acids, such as lithocholic acid (LCA), deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) [23,31,32]. However, during the progression of ICP, placental function is severely disrupted by accumulative bile acids. In turn, compromised trophoblasts are impaired to fulfill their role in maintaining bile acid homeostasis in fetal-placental-maternal triad, which may exacerbate the cholestastic condition and enhance toxicity of bile acid to fetus. We showed here intracellular edema and cell apoptosis was increased in placenta during maternal cholestasis induced by estradiol as well as by cholic acid. Administration of FXR agonist W450 significantly attenuated intracellular edema and cell apoptosis in mouse placenta, which would maintain the normal function of trophoblast.

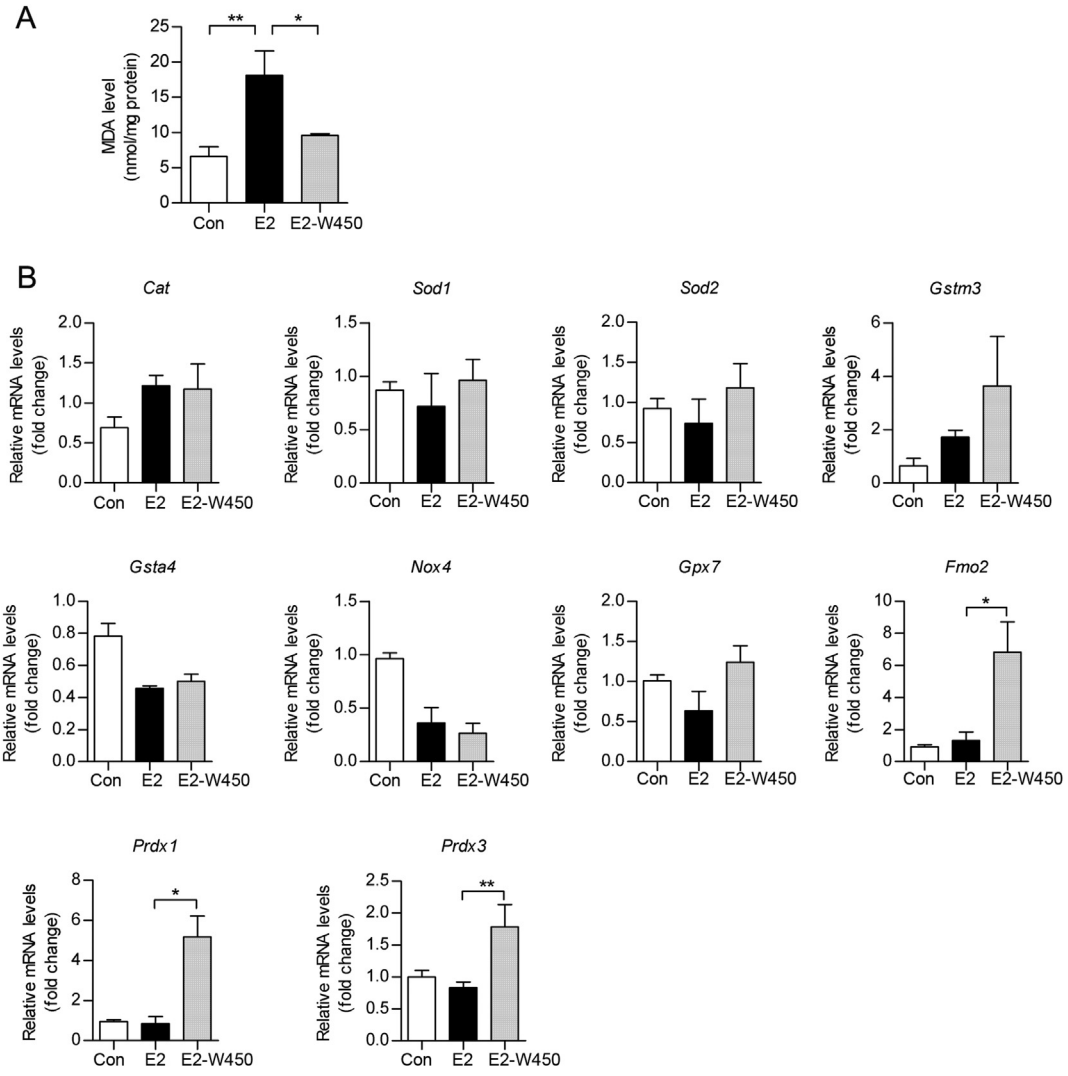

Fig. 4. Administration of W450 alleviated oxidative stress and induced genes involved in anti-oxidative response in placenta. (A) Lipid peroxidation in mouse term placentas was determined by detecting Malondialdehyde (MDA) level. (B) The mRNA expressions of anti-oxidative response genes, including Catalase (*Cat*), superoxide dismutase 1 (*Sod1*), superoxide dismutase 2 (*Sod2*), glutathione S-transferase mu 3 (*Gstm3*), glutathione S-transferase alpha 4 (*Gsta4*), NADPH oxidase 4 (*Nox4*), glutathione peroxidase 7 (*Gpx7*), flavin containing monooxygenase 2 (*Fmo2*) peroxiredoxin 1 (*Prdx1*) , and peroxiredoxin 3 (*Prdx3*) in mouse term placentas were determined by qRT-PCR. (n = 6–8, *p < 0.05, **p < 0.01, Student's t-test).

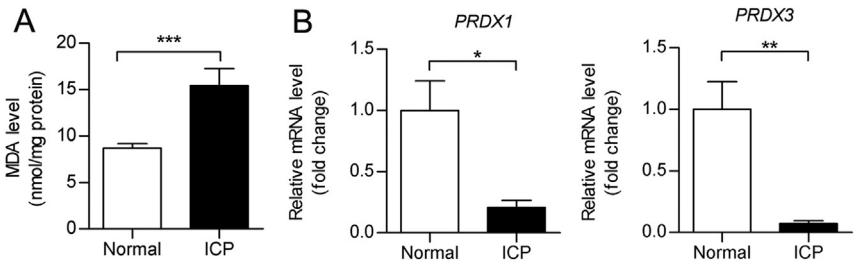

Fig. 5. Elevated oxidative stress level in human placentas from ICP patients. (A) Lipid peroxidation in human term placentas from normal pregnancies (normal, n = 20) and ICP patients (ICP, n = 11) was determined by malondialdehyde (MDA) assay. (B) Total RNA from human placenta was subjected to qRT-PCR for the determination of peroxiredoxin 1 (*PRDX1*), and peroxiredoxin 3 (*PRDX3*) mRNA expression. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Student's t-test).

Currently, the treatment of ICP with the use of ursodeoxycholic acid (UDCA) S-adenosymethionine (SAMe), dexamethasone and cholestyramine is predominantly based on clinical symptoms [7,33]. Among them, UDCA is the most commonly used and appeared to be the most efficient one [1,12]. UDCA is a polar bile acid, which functions through decreasing the hydrophobicity and toxicity of the bile pool [34]. Studies reported that UDCA was effective in ameliorating pruritus and improving liver test in patients with ICP, although the size of the benefit is small [35,36]. On the other hand, it was also suggested that there was insufficient evidence to recommend UDCA to improve fetal outcomes [12,33]. In the meta-analysis of randomized controlled trials concerning UDCA versus placebo in treating ICP [37], UDCA was not found to be significantly better than placebo in reducing severity of pruritus [38,39]. In addition, *Titta Joutsiniem*i et al. demonstrated that UDCA treatment improve itching in ICP but there were not significant differences in total BA level [35]. Furthermore, in a rat model of cholestasis induced by bile duct obstruction during pregnancy, administration of UDCA partly prevented placental oxidative stress, but failed to correct hypercholanemia [29]. More importantly, *Liu* et al. demonstrated that UDCA was less effective in lowering bile acids and afforded less hepatoprotection than FXR agonist GW4064 in rat models of cholestasis [40]. FXR, also known as a bile acid receptor, was found to be expressed in human placenta with low level [23,24]. As an endogenous sensor for bile acids, FXR regulates a profile of genes involved in bile acid biosynthesis, conjugation, and transport [16–18]. FXR ligands have been shown to be effective in treating many liver disorders, including non-alcoholic fatty liver, alcoholic liver disease, gallstone disease and cholestastic liver disease [41,42]. UDCA is not an FXR agonist. Potent and selective agonists of FXR, such as GW4064 and 6-ECDCA, are more effective in lowering bile acid level than UDCA in cholestastic diseases [43]. In this study, we demonstrated that W450, a ligand of FXR with high affinity and efficiency [44], was able to reduced circulatory and hepatic bile acid level in a model of E2 induced maternal cholestasis. Induction of bile acid transporters *Abcb11*, *Abcc2* and *Abcb4* in mouse placenta by W450 would alleviate bile acid overload and be beneficial for fetus. The role of W450 in ameliorating pruritus during ICP still needs further investigation.

In addition to lowering bile acid level in ICP model, we also explored other mechanisms. PRDX1 and PRDX3 belong to a ubiquitous family of antioxidant enzymes, named peroxiredoxins, which are known to scavenge reactive oxygen species and protect cells against oxidative stress [25,26]. Mammalian PRDX1 is a multifunctional protein, which is abundantly expressed and originally identified as an intracellular scavenger of H_2O_2 [26]. Especially, PRDX3 was reported to predominantly act within the mitochondrial matrix and play an essential role in placental antioxidant defense [27]. PRDX1 and PRDX3 function through an oxidation–reduction cycle on their catalytically active cysteine sites. Here we showed that activation of FXR by W450 induced *Prdx1* and *Prdx3* expression which may contribute to protective effect of FXR against maternal cholestasis induced oxidative stress in placenta. We demonstrated the expression of *Fmo2* in mouse placenta was increased after W450 treatment whereas no significant change of FMO2 level was indicated comparing normal and ICP placentas. FMO2 was reported to participate in drugs and ROS metabolism in mammals [45]. However, FMO2 is expressed as a non-functional truncated protein in some human populations [45]. It is possible that upregulation of *Fmo2* in mice was mediated by drug itself.

In summary, our data indicated that W450 induced activation of placenta FXR, which maintained placental homeostasis via two possible mechanisms, by promoting bile acid transport, and by protecting against oxidative stress through induction of antioxidant, such as *Prdx1* and *Prdx3*. Thus, our results support the notion that potent FXR agonists might represent promising specific drugs for treating ICP.

Conflict of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2015.02.005.

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