Original Article

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Impaired Compensatory Vasodilatory Effect Mediated by Wolfram Syndrome 1 and Corticotropin-Releasing Hormone Family Peptides in 17α-Ethynylestradiol-Induced Intrahepatic Cholestasis Pregnant Rats When Under Additional Acute Hypoxia Stress

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Abstract

Objective: To investigate the possible regulatory mechanism of corticotropin-releasing hormone (CRH), urocortin (UCN), and Wolfram syndrome 1 (WFS1) in 17α-ethynylestradiol (EE)-induced intrahepatic cholestasis pregnant rats and its ischemia reperfusion (IR) model.

Methods: Pregnant rats (n=60) were randomly divided into four experimental groups by random number table (Control, EE, IR, and EE-IR groups),and were studied on the 17th, 19th, and 21st gestational days (GD) (n=5 in each group at the indicated time). Growth and development indicators of fetal rats among these four groups were recorded. Enzyme-linked immunosorbent assay was employed to detect CRH, UCN, and WFS1 levels in maternal sera. Western blotting and real-time polymerase chain reaction were used to quantify placental protein and placental mRNA levels of CRH, UCN, and WFS1. Multivariate analysis of variance and least significant difference test were used to establish the group and individual comparisons.

Results: A significant difference was found in placenta weight (F=8.10, P<0.05), fetal rat weight (F=40.86, P<0.05), fetal rat length (F=61.61, P<0.05), and fetal rat tail length (F=55.63, P<0.05) among four groups on the 17th ,19th , and 21st GD.What's more, the overall differences of maternal serum UCN levels among Control, EE, IR, and EE-IR groups were significant (F=2.48, P<0.05). Expression of WFS1 mRNA in the EE-IR group was significantly increased and higher than Control (0.46±0.15 *vs.* 0.24±0.09, P<0.05), EE (0.46±0.15 *vs.* 0.17±0.04, P>0.05), and IR (0.46±0.15 *vs.* 0.22±0.15, P>0.05) groups at 19th GD, indicating that endoplasmic reticulum stress may be activated. However, the expression of CRH (0.42±0.05 *vs.* 0.58±0.12, P<0.05), UCN (0.43±0.01 *vs.* 0.47±0.16, P>0.05), and WFS1 (0.57±0.07 *vs.* 0.74±0.12, P>0.05) protein in the EE-IR group was subsided compared to the IR group at 17th GD.

Conclusion: Fetal rat growth restriction was found in the EE-induced intrahepatic cholestasis model. This study revealed that significant changes in the maternal sera level of UCN , placental level of WFS1 mRNA and placental levels of CRH, UCN, and WFS1 protein in chronic versus acute stress in a rat model of pregnancy. This suggests an impaired compensatory vasodilatory effect mediated by these factors at gene transcription and protein translation levels, following acute hypoxia stress in EE-induced intrahepatic cholestasis in pregnant rats.

Keywords: Cholestasis, intrahepatic; Pregnancy; Compensatory vasodilatory effect; WFS1; CRH family peptides; Ischemia-reperfusion

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific disease characterized by maternal pruritus, icterus, increased maternal serum total bile acid (TBA), and modestly elevated serum transaminase levels during pregnancy. These changes disappear quickly after birth but often recur in subsequent pregnancies. The incidence of this disorder varies by population, region, and season,[1,2] and the etiology and pathogenesis of ICP are yet to be fully elucidated.

In physiological conditions, the placenta contains 50% blood oxygen volume reserve with only 50% of villous cavity blood flow being involved in the fetal blood-oxygen exchange.[3] Interestingly, it has been reported that ICP patients have a 29% reduction in placental lobular villi vascular volume, suggesting placental insufficiency.[4] The mechanism of blood flow regulation in the human utero-placental-fetal (UPF) unit is characterized by high

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blood flow volume, low vascular resistance, and lack of neural regulation.[5,6]

The process is efficient and accurate[7] and mainly depends on circulatory and/or local vasodilatory factors. Vasodilatory factors include prostaglandin I_2 and corticotropin-releasing hormone (CRH) family of peptides,[5] the latter mainly including CRH and urocortin (UCN). Human and ovine CRH vasodilatory effects were 53 times more potent than those of prostaglandin I_2[8]; similarly, the UCN vasodilatory effect was approximately 30-fold higher than that of CRH.[9] Hence, the CRH family of peptides is one of the most important vasoactive factors to regulate placenta-fetal blood circulation through their concentration-dependent compensatory vasodilatory effect. The endoplasmic reticulum (ER) is the major organelle for polypeptide biosynthesis. Hence, the CRH family of peptides of pregnant rats was mainly biosynthetic in placental ER to regulate placenta-fetal blood circulation.

ICP is associated with several adverse perinatal outcomes, including meconium-stained amniotic fluid, fetal hypoxia with premature delivery, and sudden intrauterine fetal death.[10–12] Some literature showed that TBA levels higher than 40 mol/L increased the risk of pregnancy complications such as asphyxial events, spontaneous preterm deliveries, meconium-stained amniotic fluid, and neonatal respiratory distress.[13–15] Moreover, sudden intrauterine fetal death in ICP was associated with acute intrauterine fetal hypoxia,[16] which might be related to blood flow dysregulation of the UPF unit.[17]

Ischemia, hypoxia, hyperglycemia, and lipid or protein overload cause imbalanced ER homeostasis,[18,19] leading to ER stress. ER stress is either acute or chronic in nature and is manifested as distinct cell survival or death outcomes.[20,21] Hypoxia and/or ischemia have been reported to induce acute ER stress which is typical of short duration (minutes to hours).[20] Chronic ER stress can persist for several days to years and is often a pro-survival, adaptive mechanism.[20] However, when ER stress persists, cellular survival mechanisms are overcome by ER stress-mediated apoptotic cell death.[22] Wolfram syndrome 1 (WFS1) protein is an ER-resident membrane glycoprotein.[23] Research has shown that WFS1 deficiency could activate the ER stress pathway.[22] WFS1 plays a key role in regulating ER stress to maintain ER homeostasis by affecting both, gene transcription and protein translation.[24] WFS1 protein is highly expressed in placental cells.[25] CRH and UCN are mainly synthesized in the ER of decidua, myometrium, and placental villous syncytiotrophoblasts during pregnancy.[5,26]

Given the hypoxic stress underlying ICP, we employed rat models of 17α-ethynylestradiol (EE)-induced intrahepatic cholestasis and ischemia-reperfusion (IR) during pregnancy to mimic acute intrauterine fetal hypoxia in ICP. In our study, subcutaneously injecting EE daily for 5 consecutive days could activate chronic ER stress and the IR model would activate acute ER stress. We hypothesize that acute fetal hypoxia in patients with ICP activates acute ER stress, thereby impacting CRH, UCN, and WFS1 levels in the mother. If the stress persists, we postulate that the physiological adaptive changes will ultimately result in cell death and adverse perinatal outcomes. Hence, in the current study, we examined CRH, UCN, and WFS1 levels in maternal sera and the placenta. Our results shed light on the contribution of these critical factors in mediating stress responses during pregnancy, with implications for the pathogenesis of UPF hypoxia.

Materials and methods

This study was approved by the ethical committees at the West China Second University Hospital, Sichuan University (Animal Ethics Approval No. 85, 2019). All rats received humane care according to the criteria prepared by the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Animals

All Sprague–Dawley rats were purchased from Chengdu Dashuo Experimental Animal Co., Ltd (Chengdu, China). These rats were housed in a standard controlled room under a constant 12 hours light/dark cycle, ad libitum access to water and diet, humidity (40%–70%), and temperature (15 °C–20 °C). Adult female rats weighing 250–300 g were mated with adult male Sprague–Dawley rats weighing 300–350 g with a 1:1 ratio. Vaginal plugs, indicating the onset of pregnancy, were checked every morning. The day we observed a vaginal plug was recorded as the first day of gestation. The pre-pregnancy body weight of rats (the day before the observation of vaginal plug) was measured and recorded.

Grouping

Pregnant rats ($n=60$) were randomly divided into four experimental groups by random number table: Control, EE, IR, and EE-IR groups and were studied on the 17th, 19th, and 21st gestational days (GD) ($n=5$ in each group at the indicated time).

Model of intrahepatic cholestasis and IR

17α-EE was purchased from Sigma-Aldrich (Product No: E4876, St Louis Missouri, USA). Intrahepatic cholestasis in pregnant rats was induced by subcutaneously injecting EE daily (2.5 mg/kg body weight, propylene glycol as a solvent, the concentration of EE was 1 mg/mL) for 5 consecutive days starting on 14th GD.[27] Control rats were subcutaneously administered propylene glycol daily (2.5 mL/kg body weight) for 5 consecutive days starting on 14th GD (Fig. 1A).

IR model was used to mimic acute intrauterine fetal hypoxia of the UPF unit. It was induced by occluding both utero-ovarian arteries for 20 minutes and then releasing the occlusion and restoring circulation for 30 minutes before collecting samples.[28] For pregnant rats with no IR model, the procedure was the same as the IR model except that the bilateral uterus was exposed to the abdominal cavity and covered with warm (37 °C) saline-soaked gauze for 50 minutes before collecting samples to avoid the interference of time factor. Much

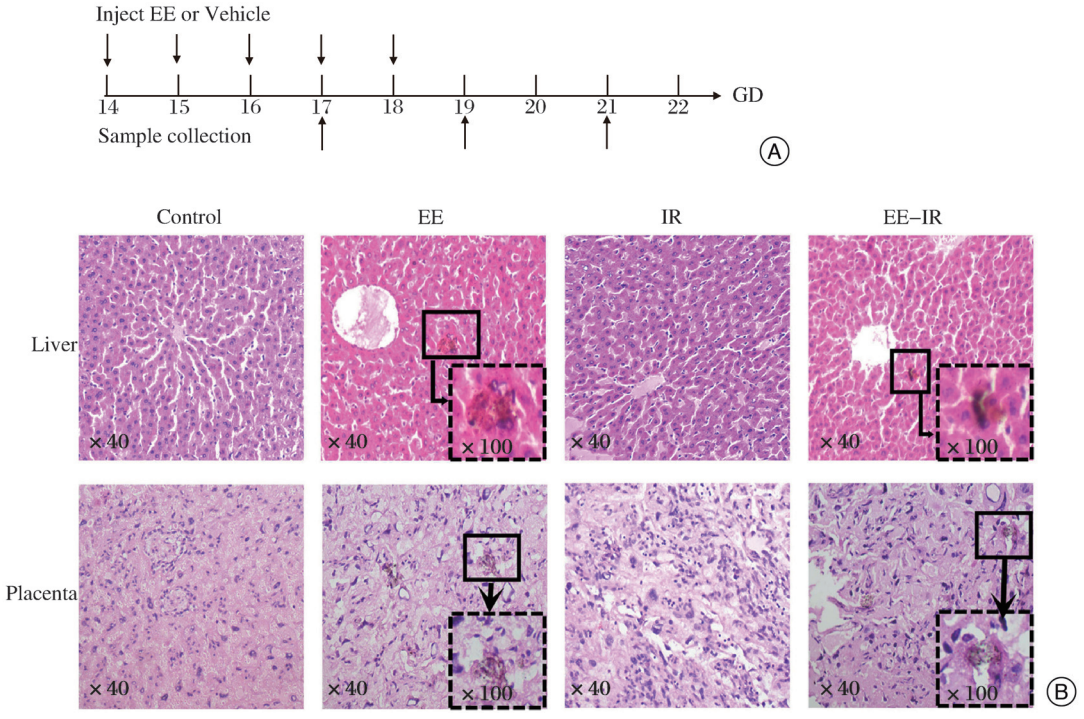

Figure 1. Establishment of pregnant rats model and pathological changes in the liver and placentae. A The diagram illustrates the establishment of EE-induced intrahepatic cholestasis in pregnant rats, IR model, and sample collection at the indicated time points. IR and EE-IR groups received similar injections except for added IR models before sample collection. The IR model was induced by occluding both utero-ovarian arteries for 20 minutes, then releasing the occlusion and restoring circulation for 30 minutes. B H&E stained sections showed pathological changes in the maternal liver and placentae in 21st GD in pregnant rats (40× magnification). Inserted images with the dotted line are higher-power images of bile plugs (100× magnification). EE: 17α-Ethynylestradiol; GD: Gestational day; H&E: Hematoxylin-eosin; IR: Ischemia-reperfusion.

more detailed information of these included groups was as follows:

Control: injected propylene glycol, and covered with warm (37 °C) saline-soaked gauze for 50 minutes.
EE (mimic ICP): injected EE, and covered with warm (37 °C) saline-soaked gauze for 50 minutes.
IR (mimic acute intrauterine fetal hypoxia in normal condition): injected propylene glycol, and induced IR model.
EE-IR (mimic acute intrauterine fetal hypoxia in ICP): injected EE, and induced IR model.

Sample collection

Surgical procedures were performed on the 17th (the day after the fourth dose of EE or propylene glycol injection), 19th (1 day after the last dose of EE or propylene glycol injection), and 21st GD (3 days after the last dose of EE or propylene glycol injection) in each group (Fig. 1A). No animals were delivered before 21st GD. Animals were anesthetized with a single dose of 10% chloral hydrate (400 mg/kg body weight, intraperitoneally).[29] A middle abdominal incision was made rapidly, and then the IR model was conducted.

The number of fetal rats per litter, meconium-stained amniotic fluid (the amniotic fluid of fetal rat was discolored), intrauterine fetal demise, and other complications were recorded. Fetal rats' body weights, body length, and tail length (all fetuses per litter), and placental weight (all placentas per litter) were measured and recorded. Placenta tissue (excluding membranes) was collected in sterile microtubes. Maternal blood was centrifuged to obtain maternal serum after incubation on ice for 15 minutes. All samples were stored at −80 °C until the time of analysis.

A small piece of liver tissue (about 2 cm × 1 cm × 0.5 cm) was randomly selected and one placenta from each pregnant rat was placed in formaldehyde for histological examination. All animals were sacrificed by exsanguination at the end of each surgical procedure.

Serum biochemical analysis

Maternal serum alanine transaminase (ALT), aspartate transaminase (AST), and TBA levels were detected by Siemens-advia® chemistry kits using a fully automatic biochemical analyzer (Siemens-advia® 2400, Germany) after centrifuging at 1000 × *g* for 10 minutes at 4 °C.

Histological examination

Pathological sections of pregnant rat livers and placentae were performed by hematoxylin-eosin (H&E) staining and observed under a microscope (Olympus, Tokyo, Japan, BX53) to view and obtain the histological image.

Enzyme-linked immunosorbent assay analysis

Maternal serum CRH, UCN, and WFS1 levels of pregnant rats were tested using a specific commercially available enzyme-linked immunosorbent assay kit (DL-develop,

Cat. No.: DL-WFS1-Ra, DL-CRH-Ra, DL-UCN-Ra, Canada-China) according to kit instructions.

RNA isolation, reverse transcription, and quantitative real-time PCR (RT-PCR)

Randomly selected three placentae from each pregnant litter were used for RNA isolation. Total RNA was isolated using the Takara RNAiso Plus (Trizol) and subjected to reverse-transcription into cDNA using the PrimeScript^{TM} RT reagent Kit with genomic deoxyribonucleic acid (gDNA) Eraser (Takara, Japan). The RT-PCR analyses were performed in the BioRad CFX96 qPCR system with the Premix Ex Taq^{TM} (Probe qPCR) master mix reagent (Takara) in two stages: Stage 1, initial denaturation 95 °C for 30 seconds; Stage 2, PCR reaction in a 40-cycle. PCR cycling program was 95 °C for 5 seconds, 62 °C for 30 seconds (β-actin); 60 °C for 30 seconds (CRH); 58 °C for 30 seconds (WFS1); and 54 °C for 30 seconds (UCN).

The probe and primer sequences used for real-time PCR were synthesized by Ruijie Biotechnology (Shanghai, China).

(1) rat CRH: forward primer 5′-CCGCAGCCGTT GAATTTCTTG-3′, probe 5′-AGCAACCTCAGCCGATT CTGATCCGCA-3′, reverse primer 5′-GTGAGGGGCG TGGAGTTG-3′;
(2) rat UCN: forward primer 5′-GGCGAATGTGGTCC AGGATC -3′, probe 5′-TCGTCTCGTCGTTGCCGCTC GCCT-3′, reverse primer5′-GCAGCAGGTGGAAGGT GAG-3′;
(3) rat WFS1: forward primer 5′-GCAGGATGAGGAT GAAGATGAAGAC-3′, probe 5′-CCGAGGACCTGCCA CTACGCCAGAA-3′, reverse primer 5′-TGAGGGCGTT GATGTGATGG -3′;
(4) rat actin: forward primer 5′-CCTAGACTTCGAGC AAGAGA-3′, probe 5′-CCACTGCCGCATCCTCTTCC TCC-3′, reverse primer 5′-GGAAGGAAGGCTGGAAG A-3′.

Analysis of relative gene expression data was done using the $2^{-\Delta\Delta Ct}$ method. Target gene expression was normalized relative to β-actin.

Western blotting

Two hundred milligrams placental tissue was placed into a 2 mL tube containing 800 mL lysis buffer (with phenylmethane sulfonyl fluoride) and homogenized. The homogenate was centrifuged at 12,000×*g* for 5 minutes at 4 °C, and the supernatant was collected. Protein concentrations were tested using a Pierce® BCA Protein Assay Kit (NCI3225CH, Thermo Scientific, Waltham, MA, USA). Lysates (50 μg protein) were mixed with 5× sodium dodecyl sulfate-polyacrylamide gel electrophoresis loading buffer at a ratio of 4:1 and heated at 100 °C for 5 minutes to denature the protein. The sodium dodecyl sulfate-polyacrylamide gel was used to separate proteins, which were then wet-transferred onto polyvinylidene difluoride membranes. Membranes were blocked with 5% milk (dissolved in 1× Tris-Buffered Saline, 0.1% Tween (TBS-T), Tris-buffered saline buffer containing 0.05% Tween 20) for 1 hour at room temperature. Membranes were incubated with primary antibody overnight at 4 °C. Primary antibodies employed in the study were as follows: rabbit-anti CRH antibody (Abcam, No: ab8901, 1:1000, England), goat-anti UCN antibody (Santa Cruz Biotechnology, No: sc-1825, 1:500, CA, USA), rabbit-anti WFS1 antibody (Abcam, No: ab48169, 1:1000), and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (Abcam, No: G8795, 1:2000). Membranes were washed with TBS-T three times for 15 minutes and incubated with the corresponding secondary antibodies (Abcam, No: ab6721; ab6885, 1:10,000) at room temperature for 1 hour. Blots were visualized using enhanced chemiluminescence and imaged on X-ray film. Relative band densities were quantitated by densitometry using Quantity One software (Bio-Rad, CA, USA). The ratio of the target band intensities to GAPDH was obtained to quantify the relative protein expression level.

Statistical analysis

All analyses were conducted using the R software (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria). Pauta criterion (data greater than $\mu + 3\sigma$ or less than $\mu - 3\sigma$ will be rejected) was used to define and reject outliers. For continuous variables, such as the pre-pregnancy weight, fetal growth and development indicators (placental weight, fetal rat weight, tail length, and body length), maternal serum levels of ALT, AST, TBA, CRH, UCN, and WFS1, mean ± standard deviation or standard error of the mean were calculated for each group; however, for categorical variables, relative frequencies were calculated. According to the distributions of variables, multivariate analysis of variance and LSD test were used to establish the group and individual comparisons. All statistical tests were two-tailed, and P-values < 0.05 were considered statistically significant.

Results

Sixty pregnant rats were included in this study and randomly assigned into four groups: Control, IR, EE, and EE-IR groups. There was no significant difference in pre-pregnancy weight (the day before the observation of vaginal plug) among these four groups ($F = 1.35$, $P = 0.33$) (Table 1).

EE-induced intrahepatic cholestasis pregnant rats had adverse pregnancy outcomes

A significant difference was found in adverse pregnancy (i.e. fetal demise and meconium-stained amniotic fluid) outcomes among Control, IR, EE, and EE-IR groups ($P < 0.05$). Within the Control group: out of 15 pregnant rats, we obtained 199 fetal rats with a live birth percentage of 99.0% (197/199). One percent of them underwent intrauterine fetal demise (2/199), and 1.0% had meconium-stained amniotic fluid (2/199). In the treatment with EE group: out of 15 pregnant rats, we obtained 191 fetal rats with 68.1% (130/191) alive pups, 32.0% intrauterine fetal death (61/191), and 39.8% meconium-stained amniotic fluid (76/191). In the IR group, we got 206 fetal rats out of 15 pregnant rats and 92.2% (190/206) of them were alive and 7.8% (16/206) of them died. Six point three

Table 1

The pre-pregnancy rats' weight in each group (mean±*SEM*, g).

Groups	*n*	17th GD	19th GD	21st GD	*F*	*P*
Control	5	250±4	245±21	246±16	1.35	0.33
IR	5	249±16	244±12	248±24		
EE	5	257±16	246±14	260±35		
EE-IR	5	251±6	249±21	230±14		

EE: 17α-Ethynylestradiol; GD: Gestational day; IR: Ischemia-reperfusion; *SEM*: Standard error of the mean.

percent of the fetus had meconium-stained amniotic fluid in the IR group (13/206). In contrast, in the EE-IR group, out of 15 pregnant rats, we obtained 238 fetal rats with a live birth percentage of 79.0% (188/238). Twenty-one percent of them underwent intrauterine fetal demise (50/238), while 20.2% had meconium-stained amniotic fluid (48/238). It was noted that the incidence of adverse pregnancy outcomes in EE-induced model rats was higher than that in propylene glycol-induced control rats.

EE significantly affected placenta weight, body weight, body length, and tail length in fetal rats

A significant difference was found in placenta weight among Control, EE, IR, and EE-IR groups on the 17th GD, 19th GD, and 21st GD ($F=8.10$, $P<0.05$). The average placenta weight on the 19th GD was 0.14 g higher than that on the 17th GD, likewise, the average placenta weight on the 21st GD was 0.24 g higher than that on the 17th GD. Hence, we can see that GD had a significant difference in placenta weight. While the average placenta weight of EE-induced pregnant rats on the 19th GD and 21st GD only separately increased 0.004 g and 0.08 g compared to that on the 17th GD (Table 2).

There was a significant difference in fetal rat weight among Control, EE, IR, and EE-IR group groups on the 17th GD, 19th GD, and 21st GD ($F=40.86$, $P<0.05$). The average weight in fetal rats on the 19th GD was 1.20 g higher than that on the 17th GD, in addition, the average weight of the fetus on the 21st GD was 3.28 g higher than that on the 17th GD. Hence, the effect of GD on fetal rat weight was significant. However, EE-induced fetal rat on the 21st GD only increased 2.05 g compared to that on the 17th GD ($P<0.05$) (Table 2).

The overall differences of fetus rat length among Control, EE, IR, and EE-IR groups on the 17th GD, 19th GD, and 21st GD were significant ($F=61.61$, $P<0.05$). The average body length in fetal rats on the 19th GD and 21st GD was independently increased 0.97 cm and 2.00 cm compared to that on the 17th GD (Table 2). These four groups had a significant difference in fetus rat tail length on the 17th GD, 19th GD, and 21st GD ($F=55.63$, $P<0.05$). Tail length in fetal rats on the 19th GD was 0.32 cm longer than that on the 17th GD, besides, tail length on the 21st GD was 0.81 cm longer than that on the 17th GD. Though, EE-induced fetus rat tail length on the 21st GD was only increased 0.63 cm when compared to that on the 17th GD (Table 2).

EE affects liver function

There was a significant difference in maternal serum levels of ALT ($F=2.98$, $P<0.05$), AST ($F=2.07$, $P<0.05$), and TBA ($F=3.20$, $P<0.05$) among Control, EE, IR, and EE-IR groups on the 17th GD, 19th GD, and 21st GD. In addition, the level of TBA was significantly different. Serum TBA concentration in EE-induced intrahepatic cholestasis in pregnant rats was 24.80 μmol/L higher than that of propylene glycol treated rats (Table 3). Besides, placental homogenate and fetal rat homogenate levels of

Table 2

Growth and development indicators of fetal rats in each group (mean±*SEM*).

GD	Groups	*n*	Placenta weight (g)	Fetal rat weight (g)	Fetal rat body length (cm)	Fetal rat tail length (cm)
17th	Control	5	0.27±0.04	0.45±0.09	1.58±0.16	0.50±0.08
	EE	5	0.27±0.18	0.36±0.07	1.44±0.02	0.53±0.01
	IR	5	0.27±0.02	0.51±0.07	1.61±0.10	0.46±0.01
	EE-IR	5	0.23±0.05	0.42±0.07	1.54±0.10	0.48±0.04
19th	Control	5	0.41±0.04	1.64±0.16	2.55±0.11	0.82±0.10
	EE	5	0.27±0.10	1.04±0.33	2.37±0.17	0.71±0.05
	IR	5	0.40±0.05	1.80±0.38	2.59±0.19	0.88±0.06
	EE-IR	5	0.31±0.05	1.23±0.34	2.21±0.39	0.73±0.18
21st	Control	5	0.51±0.08	3.72±0.79	3.58±0.31	1.31±0.15
	EE	5	0.35±0.04	2.41±0.52	3.15±0.23	1.15±0.02
	IR	5	0.40±0.03	3.05±0.40	3.27±0.22	1.13±0.03
	EE-IR	5	0.37±0.04	2.66±0.47	3.09±0.13	1.14±0.09
F			8.10	40.86	61.61	55.63
P			<0.05	<0.05	<0.05	<0.05

EE: 17α-Ethynylestradiol; GD: Gestational day; IR: Ischemia-reperfusion; *SEM*: Standard error of the mean.

Table 3

Maternal serum biochemical parameters of pregnant rats (mean±*SD*).

GD	Groups	*n*	ALT (U/L)	AST (U/L)	TBA (μmol/L)
17th	Control	5	64.32±3.80	187.11±36.75	11.69±2.12
	EE	5	27.16±2.63	109.74±21.45	36.50±8.41
	IR	5	42.77±3.08	113.41±14.68	14.50±7.10
	EE-IR	5	50.79±14.21	275.30±40.14	16.05±5.28
19th	Control	5	46.20±14.42	124.27±59.37	6.12±1.50
	EE	5	50.02±14.31	195.27±18.10	98.08±22.10
	IR	5	44.68±16.86	149.89±10.71	17.40±9.19
	EE-IR	5	62.52±31.10	226.52±29.65	60.06±35.98
21st	Control	5	54.20±10.79	116.38±30.41	17.10±12.16
	EE	5	42.70±2.13	176.63±42.41	23.90±12.33
	IR	5	47.40±7.17	123.08±7.72	20.51±14.12
	EE-IR	5	43.76±5.49	152.70±26.28	33.68±15.24
F			2.98	2.07	3.20
P			<0.05	<0.05	<0.05

ALT: Alanine transaminase; AST: Aspartate transaminase; EE: 17α-Ethynylestradiol; GD: Gestational day; IR: Ischemia-reperfusion; *SD*: Standard deviation; TBA: Total bile acid.

TBA in EE-induced intrahepatic cholestasis pregnant rats were significantly higher than that in control rats (Table 4).

We conducted a histological examination for pathological changes in pregnant rat livers and placentae at 17th GD, 19th GD, and 21st GD. Pathological changes in the liver and placentae on these three days were similar in each respective group. Hence, figure 1B shows representative H&E-stained liver and placentae sections at 21st GD. In particular, we observed that in propylene glycol treated rats, the liver lobule, central vein, hepatic sinusoid, and hepatic cords were recognizable, while in the EE-induced intrahepatic cholestasis group, a cluster-like bile salt deposition was present in hepatic cell cords and hepatocytes (Fig. 1B top panels). There were no obvious ischemia and hypoxia-dependent changes detected in the IR model.

Similarly, we also detected a cluster-like bile salt plug in the placenta in the EE-induced intrahepatic cholestasis model (Fig. 1B bottom panels). In contrast, placenta sections were visibly normal and consistent with the pregnancy period in Control rats. No obvious ischemia and hypoxia-dependent change were detected in the IR model.

UCN levels showed dynamic changes in the maternal sera of pregnant rats

There was no significant difference in maternal serum CRH levels among Control, EE, IR, and EE-IR groups ($F=1.01$, $P=0.46$) (Fig. 2A). However, a significant difference was found in maternal serum UCN levels among Control, EE, IR, and EE-IR groups ($F=2.48$, $P<0.05$). UCN levels in the EE group were significantly decreased and were 16.59 ng/mL lower than that in the Control group ($P<0.05$). Similarly, UCN levels in the Control-IR group were 10.71 ng/mL lower than that in the Control

Table 4

Biochemical parameters of progeny related tissue in four groups (mean±*SD*).

GD	Groups	*n*	P-TBA (μmol/L)	R-TBA (μmol/L)	A-ALT (U/L)	A-AST (U/L)	A-TBA (μmol/L)
17th	Control	5	3.48±0.87	0.92±0.20	1.05±0.62	2.18±0.34	0.66±0.18
	EE	5	4.03±2.38	1.07±0.38	1.82±1.16	6.19±2.92	1.24±0.55
	IR	5	2.89±1.83	0.90±0.50	2.31±1.57	8.06±5.58	0.92±0.47
	EE-IR	5	4.51±1.00	0.94±0.11	0.89±0.32	4.24±1.55	0.75±0.13
19th	Control	5	2.52±0.48	1.74±0.44	2.24±0.30	6.50±2.16	1.16±0.31
	EE	5	18.48±10.26	3.85±1.38	7.51±3.52	116.71±91.52	14.22±5.65
	IR	5	4.45±1.82	3.26±1.33	2.50±1.02	14.79±6.04	2.35±0.96
	EE-IR	5	17.82±4.55	4.87±2.30	13.19±9.16	215.82±172.26	17.35±12.81
21st	Control	5	5.32±0.81	9.27±2.88	6.40±1.71	35.70±9.54	5.44±0.86
	EE	5	7.71±1.84	13.11±2.65	9.18±1.82	79.78±31.50	17.23±11.69
	IR	5	7.35±2.69	6.90±1.73	5.33±1.10	38.49±8.08	6.42±1.57
	EE-IR	5	15.40±3.47	14.62±4.21	15.78±8.44	156.82±114.96	19.69±2.54
F			3.49	5.94	1.56	1.09	1.97
P			<0.05	<0.05	0.15	0.39	0.06

A-ALT: Amniotic fluid level of alanine transaminase; A-AST: Amniotic fluid level of aspartate transaminase; A-TBA: Amniotic fluid level of total bile acid; P-TBA: Placental homogenate level of total bile acid; R-TBA: Fetal rat homogenate level of total bile acid; EE: 17α-Ethynylestradiol; GD: Gestational day; IR: Ischemia-reperfusion; *SD*: Standard deviation.

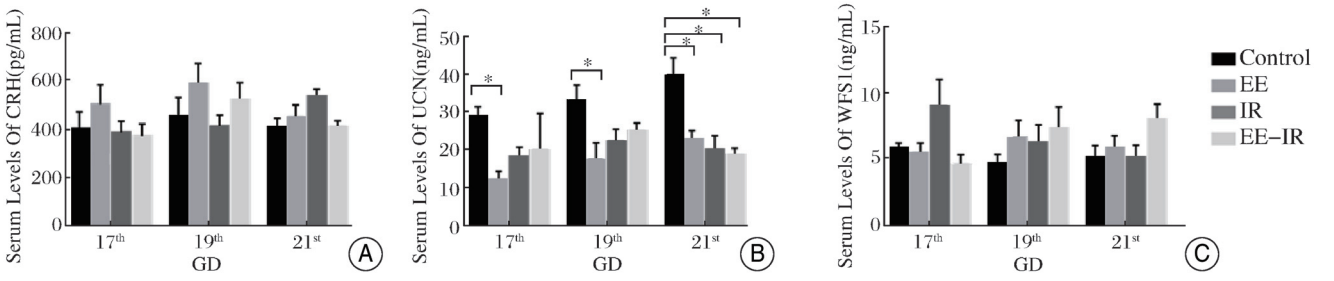

Figure 2. Dynamic serum expression of CRH, UCN, and WFS1 in pregnant rats. A Serum levels of CRH ($F=1.01$, $P=0.46$); B Serum levels of UCN ($F=2.48$, $P<0.05$); C Serum levels of WFS1 ($F=1.84$, $P=0.09$) ($n=5$ in each group at indicated time). Data are shown as mean ± *SEM*. CRH: Corticotrophin releasing hormone; EE: 17α-Ethynylestradiol; GD: Gestational day; IR: Ischemia-reperfusion; *SEM*: Standard error of the mean; UCN: Urocortin; WFS1: Wolfram syndrome 1 protein.

group ($P<0.05$). The maternal UCN level in the EE-IR group at 21st GD was significantly lower than that in the Control group (18.45 ± 1.86 *vs.* 39.31 ± 4.98, $P<0.05$) (Fig. 2B). While no significant difference was found in maternal WFS1 levels among Control, EE, IR, and EE-IR groups ($F=1.84$, $P=0.09$) (Fig. 2C) (Table S1, http://links.lww.com/MFM/A13).

Placental CRH, UCN, and WFS1 mRNA expression

CRH mRNA expression did not significantly vary among Control, EE, IR, and EE-IR groups ($F=0.94$, $P=0.52$) (Fig. 3A). Similarly, no significant difference was found in UCN mRNA expression among Control, EE, IR, and EE-IR groups ($F=1.19$, $P=0.33$) (Fig. 3B). However, there was a significant difference in placental WFS1 mRNA expression among Control, EE, IR, and EE-IR groups ($F=2.97$, $P<0.05$) (Fig. 3C). Under stress, WFS1 mRNA in the EE group and IR group was decreased and lower than Controls at 17th GD, 19th GD, and 21st GD, but the difference was not statistically significant. However, WFS1 mRNA expression in the EE-IR group was significantly increased and higher than Control (0.46 ± 0.15 *vs.* 0.24 ± 0.09, $P<0.05$), EE (0.46 ± 0.15 *vs.* 0.17 ± 0.04, $P>0.05$), and IR (0.46 ± 0.15 *vs.* 0.22 ± 0.15, $P>0.05$) groups at 19th GD, besides, WFS1 mRNA expression at 21st GD in the EE-IR group was higher than Control (0.32 ± 0.11 *vs.* 0.25 ± 0.10, $P>0.05$), EE (0.32 ± 0.11 *vs.* 0.24 ± 0.07, $P>0.05$), and IR (0.32 ± 0.11 *vs.* 0.24 ± 0.07, $P>0.05$) groups, but the difference was not statistically significant (Table S2, http://links.lww.com/MFM/A14).

Placental CRH, UCN, and WFS1 protein expression

A significant difference in placental CRH was found among Control, EE, IR, and EE-IR groups ($F=6.45$, $P<0.05$). CRH in the EE group increased and was higher than that in the Control group on the 17th GD (0.36 ± 0.06 *vs.* 0.30 ± 0.06, $P>0.05$), 19th GD (0.29 ± 0.07 *vs.* 0.14 ± 0.05, $P>0.05$), and 21st GD (0.34 ± 0.01 *vs.* 0.29 ± 0.03, $P>0.05$). In ischemia-hypoxia, CRH in the IR group was significantly higher than that in the Control group on the 17th GD (0.58 ± 0.12 *vs.* 0.30 ± 0.06, $P<0.05$) and 19th GD (0.41 ± 0.07 *vs.* 0.14 ± 0.05, $P<0.05$), similarly, it was also significantly higher than that in the EE group at 17th (0.58 ± 0.12 *vs.* 0.36 ± 0.06, $P<0.05$). The levels of placental CRH protein in the EE-IR group were decreased and lower than that in the IR group at 17th GD (0.42 ± 0.05 *vs.* 0.58 ± 0.12, $P<0.05$) and 21st GD (0.33 ± 0.01 *vs.* 0.40 ± 0.02, $P>0.05$) (Fig. 4A).

Similarly, there was a significant difference in placental UCN among Control, EE, IR, and EE-IR groups ($F=4.69$, $P<0.05$). UCN in the EE group was increased and significantly higher than that in the Controls on 21st GD (0.32 ± 0.08 *vs.* 0.15 ± 0.02, $P<0.05$). In ischemia-hypoxia stress, UCN in the IR group was significantly higher than that in the Control group on 17th (0.47 ± 0.16 *vs.* 0.11 ± 0.05, $P<0.05$), 19th (0.28 ± 0.12 *vs.* 0.17 ± 0.03, $P>0.05$), and 21st GD (0.29 ± 0.08 *vs.* 0.15 ± 0.02, $P<0.05$), similarly, it was also significantly higher than that in the EE group on 17th (0.47 ± 0.16 *vs.* 0.26 ± 0.07, $P<0.05$) and 19th GD (0.28 ± 0.12 *vs.* 0.25 ± 0.12, $P<0.05$). UCN in the EE-IR group was upregulated and significantly higher

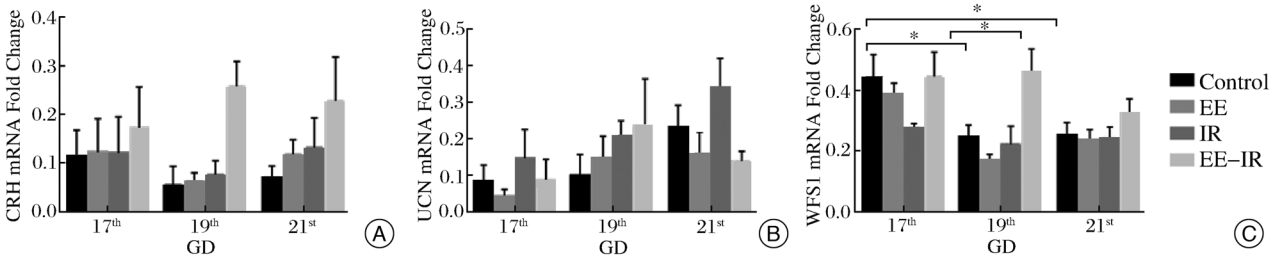

Figure 3. Expression of CRH, UCN, and WFS1 mRNA in placentae of pregnant rats. A Quantitative analysis of CRH mRNA expression normalized to β-actin in pregnant rats placentae ($F=0.94$, $P=0.52$); B Quantitative analysis of UCN mRNA expression ($F=1.19$, $P=0.33$); C Quantitative analysis of WFS1 mRNA expression ($F=2.97$, $P<0.05$, $n=5$ in each group at indicated time and three placentae were randomly selected in each litter, $^{*}P<0.05$). Data are shown as mean ± *SEM*. CRH: Corticotrophin releasing hormone; EE: 17α-Ethynylestradiol; GD: Gestational day; IR: Ischemia-reperfusion; *SEM*: Standard error of the mean; UCN: Urocortin; WFS1: Wolfram syndrome 1 protein.

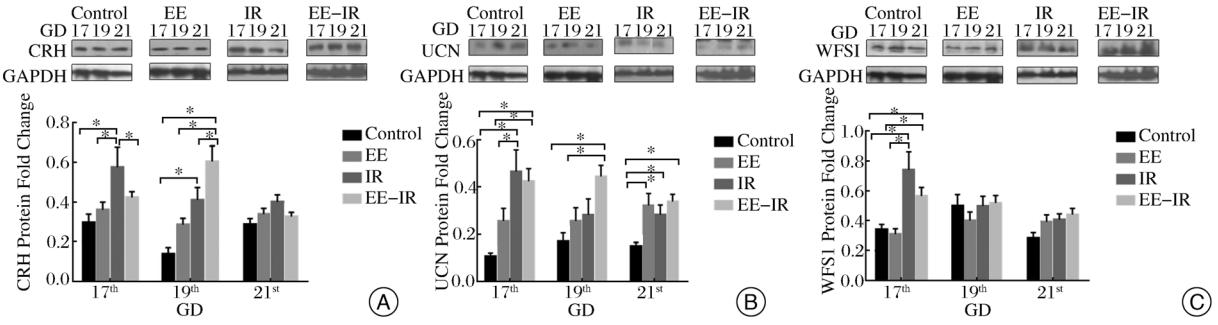

Figure 4. CRH, UCN, and WFS1 protein levels in pregnant rats placentae. A Representative western blots and densitometric analysis of protein CRH expression normalized to GAPDH ($F=6.45$, $P<0.05$); B Representative western blots and densitometric analysis of protein UCN expression normalized to GAPDH ($F=4.69$, $P<0.05$); C Representative western blots and densitometric analysis of protein WFS1 expression normalized to GAPDH ($F=4.37$, $P<0.05$). ($n=5$ in each group at the indicated time and three placentae were randomly selected in each litter, $^{*}P<0.05$). Data are shown as mean ± *SEM*. CRH: Corticotrophin releasing hormone; EE: 17α-Ethynylestradiol; GD: Gestational day; IR: Ischemia-reperfusion; *SEM*: Standard error of the mean; UCN: Urocortin; WFS1: Wolfram syndrome 1 protein.

than both, EE (0.43 ± 0.01 *vs.* 0.26 ± 0.07, $P<0.05$) and Control (0.43 ± 0.01 *vs.* 0.11 ± 0.05, $P<0.05$) groups at 17th GD, and this trend was similar at 19th GD and 21st GD, but its expression had no significant difference with the IR group (Fig. 4B).

Placental WFS1 significantly varies among Control, EE, IR, and EE-IR groups ($F=4.37$, $P<0.05$). WFS1 expression in the IR group (0.74 ± 0.12 *vs.* 0.31 ± 0.01, $P<0.05$) and EE-IR group (0.57 ± 0.07 *vs.* 0.31 ± 0.01, $P<0.05$) was significantly higher than that in the EE on the 17th GD. Similarly, WFS1 expression in the IR group (0.74 ± 0.12 *vs.* 0.34 ± 0.05, $P<0.05$) and EE-IR group (0.57 ± 0.07 *vs.* 0.34 ± 0.05, $P<0.05$) was higher than that in the Control group. While WFS1 expression in the EE-IR group was lower than that in the IR group on 17th GD (0.57 ± 0.07 *vs.* 0.74 ± 0.12, $P>0.05$) (Fig. 4C) (Table S3, http://links.lww.com/MFM/A15).

Discussion

In our study, we used an animal model to mimic ICP and acute intrauterine fetal hypoxia. EE and IR were separately used to induce chronic stress and acute ischemia-hypoxia stress. Pregnant rats in the EE-induced intrahepatic cholestasis group showed significant differences in placental weight, fetal weight, and fetal body length, and tail length indicative of fetal growth restriction. Expressions of WFS1 mRNA in the EE-IR group were increased and higher than Control, EE, and Control-IR groups, indicating that ER stress may be activated. However, under ischemia-hypoxia stress, the expression of CRH family peptides and WFS1 protein was subsided in EE-induced intrahepatic cholestasis compared to propylene glycol-treated control rats. These disrupted expressions of CRH, UCN, and WFS1 at gene transcription and protein translation levels implied impaired compensatory response to hypoxia stress in EE-induced intrahepatic cholestasis in pregnant rats.

There are several methods to induce intrahepatic cholestasis model including obstructive cholestasis and drug-induced cholestasis, and the latter was widely used for ICP. We reproduced EE-induced intrahepatic cholestasis rats regarding Crocenzi's methods.[27] However, finally, we subcutaneously administrated pregnant rats with EE 2.5 mg/kg body weight per day instead of with 5 mg/kg because of the high mortality rate. We hypothesized that this high mortality rate of fetal rats may be associated with drug-induced damage. Chang *et al.*[30] found that at 5 mg/kg body weight per day, EE-induced intrahepatic cholestasis rats result in liver injury (liver damage and large areas of inflammatory cell infiltration), which substantiates our hypothesis.

In the present study, histological analyses of pregnant rat liver sections revealed bile salt crystallization/deposits without any structural abnormalities in the EE and EE-IR model, which is typically observed in liver biopsies of patients with ICP.[31] In addition, we also observed the deposition of bile salts in the placentae of the EE-induced intrahepatic cholestasis pregnant rat. Moreover, maternal serum TBA levels and placental homogenate TBA levels were increased in EE-induced intrahepatic cholestasis pregnant rats and significantly higher than that in propylene glycol treated control rats. Besides, the average maternal weight gain in EE-induced pregnant rats was less than that in propylene glycol treated rats during pregnancy. What's more, the rate of adverse pregnancy outcomes in EE-induced cholestasis pregnant rats was higher than that in the propylene glycol treated control rats. Thus, the EE-induced ICP model in our study was reproduced successfully. The reason why we can see a cluster-like bile salt plug in the placenta in the EE and EE-IR model is that bile acid and bile salt can transfer across the rat placenta-maternal liver tandem.

It is well-established that sudden intrauterine fetal death in ICP is related to acute intrauterine fetal hypoxia.[32] Thus, in the present study, we used an IR model to mimic acute intrauterine fetal hypoxia aimed to investigate a potential regulatory mechanism in response to acute UPF hypoxia in ICP. IR model will cause acute UPF hypoxia, then activate acute ER stress which can persist several minutes to hours. However, chronic ER stress can persist for several days to years, so, daily injecting EE consecutive 5 days in our study can activate chronic ER stress.

Maternal serum WFS1, placental WFS1, and placental WFS1 mRNA level in the EE group had no significant difference compared to the Control group. However, placental WFS1 protein in the IR group increased and was higher than that in control at 17th GD. Expressions of

placental WFS1 mRNA in the EE-IR group were increased and higher than that in Control, EE, and Control-IR groups. This suggests that there is a feedback regulation between WFS1 and ER stress whereby WFS1 is activated and upregulated in response to acute ER stress and to maintain cellular homeostasis when intrahepatic cholestasis was induced.

It had been reported that the placenta secretes CRH family peptides into maternal circulation increased maternal plasma levels.[33] CRH and UCN mainly participate in regulating UPF unit blood flow to maintain its physical protection role when the feto-placental unit encounters hypoxic stress. In our study, placental UCN in the EE, IR, and EE-IR group was upregulated and significantly higher than Control rats. Besides, we also found that maternal sera UCN gradually increased from 17th GD to 21st GD in the control group, while, it decreased and was significantly lower than that in EE, IR, and EE-IR groups. This implies that it is a physiological adaptation to give priority to the placenta and other vital organs under acute or chronic stress. Besides, in our study, placental UCN and CRH increased under acute stress and higher than that under chronic stress. However, maternal UCN significantly decreased, and no significant difference in maternal sera CRH was observed among these four groups. It seems that UCN exerts a more vital role than CRH in acute ischemia-hypoxia stress. We speculate that this phenomenon is a physical compensatory mechanism because the UCN vasodilatory effect was more potent than that of CRH. These results imply that CRH and UCN may exist efficient feedback and compensatory mechanism in UPF unit vasodilation to maintain its physical protection role to adapt to acute hypoxia stress. These results were in accordance with Kozicz *et al.*[34] who found that UCN plays a particularly important role in stress adaptation, and La Marca-Ghaemmaghami *et al.*[33] who investigated that chronic maternal stress may affect feto-placental CRH secretion.

Because CRH and UCN are proteins synthesized in the ER, this compensatory response could also regulate CRH and UCN expression to ensure proper UPF blood flow. In addition, WFS1, an ER-resident membrane glycoprotein, is highly expressed in placental cells and plays a key role in regulating ER stress to maintain ER homeostasis. Our results indicate that maternal serum and placental CRH, UCN, and WFS1 in the EE-induced chronic stress group were significantly higher than that in the Control group, further confirming the existence of these physical compensatory responses. However, compensatory responses in the EE-induced intrahepatic cholestasis in pregnant rats may be impaired if this chronic stress persists, and is exacerbated by additional exposure to acute hypoxia (EE-IR group), leading to disruption of ER homeostasis, which in turn cause cell death and adverse perinatal outcomes. In our study, the incidence of adverse pregnancy outcomes (ie, fetal demise and meconium-stained amniotic fluid) in the EE-IR group was higher than the EE group, which also testify to our hypothesis.

For intrahepatic cholestasis pregnant rats induced by EE under acute ischemia-hypoxia stress (EE-IR group), we found that placental WFS1 protein expression was significantly reduced, while placental WFS1 mRNA expression was increased. Moreover, placental CRH and UCN were limited in the EE-IR group when compared to the IR group. These results show that the disrupted ER homeostasis may be the underlying cause of dysfunction of WFS1 at both transcription and translation levels, accompanied by the disruption in CRH and UCN levels. Together, abnormal expression of CRH, UCN, and WFS1 may result in impaired UPF blood flow of intrahepatic cholestasis pregnant rats under acute hypoxia stress, which in turn trigger adverse pregnancy outcomes such as sudden intrauterine fetal death, fetal hypoxia, and stillbirths.

While several studies have addressed the role of ER stress and WFS1 in obstetric complications,[35,36] there is no report on WFS1 and CRH family peptides dysfunction in ICP pathophysiology. To the best of our knowledge, we are the first to report WFS1 and CRH family peptides and their impediments in rodent models of EE-induced intrahepatic cholestasis and IR. Another major strength of our study is that we are the first one to reproduce the IR model of EE-induced intrahepatic cholestasis in pregnant fetal rats to simulate fetal acute oxidative damage, providing a new research direction and new research model for ICP and fetal hypoxic stress in the future. Our research also remedies the disadvantage that placental tissue in ICP could not be obtained in early and mid-pregnancy. Future lines of investigation will expand on the role of ER stress in ICP etiogenesis and explore the molecular and signaling pathways that are affected by this disease.

In conclusion, our study revealed an impaired expression of CRH, UCN, and WFS1 in EE-induced intrahepatic cholestasis during pregnancy in rats when exposed to additional IR injury. This abnormal phenomenon implies an attenuated compensatory vasodilatory effect to hypoxia stress in EE-induced intrahepatic cholestasis, which is of important clinical interest.

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Author Contributions

Tingting Xu, Fan Zhou, and Xiaodong Wang conceived the study. Tingting Xu, Daijuan Chen, and Xixi Deng performed experiments. Tingting Xu and Yongchi Zhan analyzed data. Fan Zhou provided technical assistance. Tingting Xu drafted the manuscript. Xiaodong Wang supervised the whole study.

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Xiaodong Wang is an Editorial Board Member of *Maternal-Fetal Medicine*. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and their research groups.

References

[1] Berg B, Helm G, Petersohn L, et al. Cholestasis of pregnancy. Clinical and laboratory studies. Acta Obstet Gynecol Scand 1986;65 (2):107–113. doi:10.3109/00016348609158363.

[2] Glynn LM, Sandman CA. Evaluation of the association between placental corticotrophin-releasing hormone and postpartum depressive symptoms. Psychosom Med 2014;76(5):355–362. doi:10.1097/PSY.0000000000000066.

[3] Käär K, Jouppila P, Kuikka J, et al. Intervillous blood flow in normal and complicated late pregnancy measured by means of an intravenous 133Xe method. Acta Obstet Gynecol Scand 1980;59 (1):7–10. doi:10.3109/00016348009160077.

[4] He MM, Liu ZF, Wang XD. Decreased volume of placental lobular villi vessels in patients with intrahepatic cholestasis of pregnancy (in Chinese). Sichuan Da Xue Xue Bao Yi Xue Ban 2011;42(6):797–801. doi:10.1007/s12264-011-1035-3.

[5] Petraglia F, Imperatore A, Challis JR. Neuroendocrine mechanisms in pregnancy and parturition. Endocr Rev 2010;31(6):783–816. doi:10.1210/er.2009-0019.

[6] Boura AL, Walters WA, Read MA, et al. Autacoids and control of human placental blood flow. Clin Exp Pharmacol Physiol 1994;21 (10):737–748. doi:10.1111/j.1440-1681.1994.tb02441.x.

[7] Hansen V, Maigaard S, Allen J, et al. Effects of vasoactive intestinal polypeptide and substance P on human intramyometrial arteries and stem villous arteries in term pregnancy. Placenta 1988;9(5):501–506. doi:10.1016/0143-4004(88)90022-7.

[8] Clifton VL, Read MA, Leitch IM, et al. Corticotropin-releasing hormone-induced vasodilatation in the human fetal placental circulation. J Clin Endocrinol Metab 1994;79(2):666–669. doi:10.1210/jcem.79.2.8045990.

[9] Zhou F, Zhang L, Sun Q, et al. Expression of urocortin and corticotrophin-releasing hormone receptor-2 in patients with intrahepatic cholestasis of pregnancy. Placenta 2014;35(11):962–968. doi:10.1016/j.placenta.2014.07.019.

[10] Reyes H. Sulfated progesterone metabolites in the pathogenesis of intrahepatic cholestasis of pregnancy: Another loop in the ascending spiral of medical knowledge. Hepatology 2016;63(4):1080–1082. doi:10.1002/hep.28365.

[11] Geenes V, Chappell LC, Seed PT, et al. Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: a prospective population-based case-control study. Hepatology 2014;59(4):1482–1491. doi:10.1002/hep.26617.

[12] Wang XD, Yao Q, Peng B, et al. A clinical analysis of intrahepatic cholestasis of pregnancy in 1241 cases (in Chinese). Zhonghua Gan Zang Bing Za Zhi 2007;15(4):291–293. doi: 10.3760/j.issn:1007-3418.2007.04.013.

[13] Ataalla WM, Ziada DH, Gaber R, et al. The impact of total bile acid levels on fetal cardiac function in intrahepatic cholestasis of pregnancy using fetal echocardiography: a tissue Doppler imaging study. J Matern Fetal Neonatal Med 2016;29(9):1445–1450. doi:10.3109/14767058.2015.1051020.

[14] Glantz A, Marschall HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: relationships between bile acid levels and fetal complication rates. Hepatology 2004;40(2):467–474. doi:10.1002/hep.20336.

[15] Ovadia C, Seed PT, Sklavounos A, et al. Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy with biochemical markers: results of aggregate and individual patient data meta-analyses. Lancet 2019;393(10174):899–909. doi:10.1016/S0140-6736(18)31877-4.

[16] Wei W, Hu YY. Expression of hypoxia-regulated genes and glycometabolic genes in placenta from patients with intrahepatic cholestasis of pregnancy. Placenta 2014;35(9):732–736. doi:10.1016/j.placenta.2014.06.372.

[17] Zhang Y, Liu S, Wang X. Study on fetal hypoxia in intrahepatic cholestasis of pregnancy (in Chinese). Zhong Hua Fu Chan Ke Za Zhi 2000;35(10):600–602. doi: 10.3760/j.issn:0529-567X.2000.10.008.

[18] Jiang S, Fang QC, Jia WP. The research of WFS1 and endoplasmic reticulum stress in the pathogenesis of diabetes mellitus (in Chinese). Natl Med J China 2011;91(8):568–571. doi:10.3760/cma.j.issn.0376-2491.2011.08.018.

[19] Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 2008;7(12):1013–1030. doi:10.1038/nrd2755.

[20] Rutkowski DT, Kaufman RJ. That which does not kill me makes me stronger: adapting to chronic ER stress. Trends Biochem Sci 2007;32 (10):469–476. doi:10.1016/j.tibs.2007.09.003.

[21] Liu Y, Connor JR. From adaption to death: endoplasmic reticulum stress as a novel target of selective neurodegeneration. Neural Regen Res 2015;10(9):1397–1398. doi:10.4103/1673-5374.165227.

[22] Yamada T, Ishihara H, Tamura A, et al. WFS1-deficiency increases endoplasmic reticulum stress, impairs cell cycle progression and triggers the apoptotic pathway specifically in pancreatic beta-cells. Hum Mol Genet 2006;15(10):1600–1609. doi:10.1093/hmg/ddl081.

[23] Takeda K, Inoue H, Tanizawa Y, et al. WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. Hum Mol Genet 2001;10(5):477–484. doi:10.1093/hmg/10.5.477.

[24] Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science 2011;334(6059):1081–1086. doi:10.1126/science.1209038.

[25] Lucariello A, Perna A, Sellitto C, et al. Modulation of wolframin expression in human placenta during pregnancy: comparison among physiological and pathological states. Biomed Res Int 2014;2014:985478. doi:10.1155/2014/985478.

[26] Wang XD, Yu HY, He MM, et al. Action of corticotropin-releasing hormone on its own receptor type-1 in human placenta (in Chinese). Sichuan Da Xue Xue Bao Yi Xue Ban 2007;38(1):101–104. doi:10.3969/j.issn.1672-173X.2007.01.025.

[27] Crocenzi FA, Sánchez Pozzi EJ, Pellegrino JM, et al. Beneficial effects of silymarin on estrogen-induced cholestasis in the rat: a study in vivo and in isolated hepatocyte couplets. Hepatology 2001;34 (2):329–339. doi:10.1053/jhep.2001.26520.

[28] Okatani Y, Wakatsuki A, Shinohara K, et al. Melatonin protects against oxidative mitochondrial damage induced in rat placenta by ischemia and reperfusion. J Pineal Res 2001;31(2):173–178. doi:10.1034/j.1600-079x.2001.310212.x.

[29] Kang AJ, Zhang K, Tian F, et al. Effects of pentobarbital sodium, chloraldurat and ethylcarbamate on hematology in female SD rats (in Chinese). Chin J Comparetiva Med 2008;18(4):61–63. doi:10.3969/j.issn.1671-7856.2008.04.015.

[30] Chang MJ, Xu YJ, He WX, et al. Intestinal injury in the rat model of 17α-ethynylestradiol-induced intrahepatic cholestasis. J Dig Dis 2016;17(11):756–763. doi:10.1111/1751-2980.12407.

[31] Tomedi J. Williams Obstetrics: 23rd Edition. United States: McGraw-Hill Companies, Inc; 2010.

[32] Ozkan S, Ceylan Y, Ozkan OV, et al. Review of a challenging clinical issue: intrahepatic cholestasis of pregnancy. World J Gastroenterol 2015;21(23):7134–7141. doi:10.3748/wjg.v21.i23.7134.

[33] La Marca-Ghaemmaghami P, Dainese SM, Stalla G, et al. Second-trimester amniotic fluid corticotropin-releasing hormone and urocortin in relation to maternal stress and fetal growth in human pregnancy. Stress 2017;20(3):231–240. doi:10.1080/10253890.2017.1312336.

[34] Kozicz T. On the role of urocortin 1 in the non-preganglionic Edinger-Westphal nucleus in stress adaptation. Gen Comp Endocrinol 2007;153(1–3):235–240. doi:10.1016/j.ygcen.2007.04.005.

[35] Ma XT, Sun LZ. Relationship Between the Endoplasmic Reticulum Stress in Placenta Tissues and the Pathogenesis of Preeclampsia (In Chinese). Nan Jing Nanjing Medical University 2011;1–51.

[36] Veerbeek JHW, Tissot Van Patot MCT, Burton GJ, et al. Endoplasmic reticulum stress is induced in the human placenta during labour. Placenta 2015;36(1):88–92. doi:10.1016/j.placenta.2014.11.005.

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