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Cholestatic pregnancy is associated with reduced placental 11βHSD2 expression

M. Martineau [a,b], G. Papacleovoulou [a,d], S. Abu-Hayyeh [a,d], P.H. Dixon [a,d], H. Ji [c], R. Powrie [b], L. Larson [e], E.K. Chien [c], C. Williamson [a,d,*]

[a] Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, UK
[b] Division of Obstetric & Consultative Medicine, Women and Infants Hospital, Alpert Medical School of Brown University, Providence, RI, USA
[c] Division of Maternal-Fetal Medicine, Women and Infants Hospital, Alpert Medical School of Brown University, Providence, RI, USA
[d] Women's Health Academic Centre, Kings College London, 2nd Floor Hodgkin Building, Guy's campus, London, UK
[e] Division of Obstetric Medicine, Women's Medicine Collaborative, Alpert Medical School of Brown University, Providence, RI, USA

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ABSTRACT

Introduction: Intrahepatic Cholestasis of Pregnancy (ICP) is associated with an increased risk of fetal morbidity and mortality and is characterised by elevated bile acids in the maternal and fetal compartments. Bile acids have been shown to attenuate renal 11βHSD2 expression and, given the protective role of placental 11βHSD2 in preventing fetal exposure to excessive maternal cortisol, we aimed to establish whether raised serum bile acids in ICP influence placental 11βHSD2 expression.

Methods: Placental tissue from human and murine cholestatic pregnancy was evaluated for changes in 11βHSD2 mRNA expression compared to uncomplicated pregnancy using quantitative PCR. Parallel *in vitro* studies were performed using BeWo choriocarcinoma cells to assess the effect of different bile acid species on 11βHSD2 gene expression and whether concurrent UDCA administration can reverse any bile acid induced changes.

Results: Placental 11βHSD2 mRNA expression was reduced in human and murine cholestatic pregnancy. In BeWo cells, treatment with the primary bile acid CDCA resulted in reduced 11βHSD2 gene expression, while treatment with other primary bile acids had no significant effect. Furthermore, the tertiary bile acid UDCA, used in the treatment of ICP did not significantly affect 11βHSD2 mRNA levels either alone, or when co-administered with CDCA.

Discussion: Under cholestatic conditions placental 11βHSD2 mRNA is reduced. Studies in BeWo choriocarcinoma cells demonstrated that CDCA is likely to be the specific bile acid that mediates this effect. UDCA, the main bile acid used to treat cholestasis, did not reduce placental 11βHSD2 expression, further supporting its use in the management of ICP.

1. Introduction

Intrahepatic Cholestasis of Pregnancy (ICP) affects 0.7% of pregnancies and usually occurs after 30 weeks of gestation [1]. ICP is characterised by pruritus and biochemically by elevated maternal serum bile acids and liver enzymes. Raised serum bile acids are now considered to be the most suitable biochemical marker for the diagnosis and monitoring of the condition [2].

ICP is associated with a significantly higher incidence of fetal complications, including fetal distress, spontaneous preterm labour, meconium staining of the amniotic fluid [1–3] and intrauterine death [4]. The risk of adverse pregnancy outcome is higher if the serum bile acid concentration is ≥40 μmol/l [2,4]. Treatment with ursodeoxycholic acid (UDCA) improves maternal pruritus, serum bile acid concentrations and liver function [3,5,6]. Two recent studies suggest that UDCA treatment may also have a beneficial effect on the rate of adverse pregnancy outcomes in ICP [3,7].

In patients with chronic liver disease, raised serum bile acids inhibit renal 11 beta hydroxysteroid dehydrogenase 2 (11βHSD2) leading to sodium and water retention [8,9]. Furthermore, reduced renal 11βHSD2 mRNA expression and enzyme activity has been reported in cholestatic rodents [10,11], and *in vitro* functional

* Corresponding author. Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, UK. Tel.: +44 207 5942197.
E-mail address: catherine.williamson@ic.ac.uk (C. Williamson).

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studies demonstrate that administration of CDCA reduces 11βHSD2 enzyme activity [10,11] and increases transcriptional activation of the mineralocorticoid receptor [8].

Although morphological and gene expression changes have been described in placental tissue from pregnancies complicated by ICP [12–14], to our knowledge no studies have investigated the effect of ICP on placental 11βHSD2 expression.

In human pregnancy, maternal cortisol levels rise threefold, and are typically 5–10 times higher than fetal levels [15,16]. 11βHSD2 is a uni-directional enzyme that oxidises cortisol to the more inert glucocorticoid hormone cortisone. In the placenta, 11βHSD2 has been localised to the syncytiotrophoblast [17]. Placental 11βHSD2 gene expression progressively increases throughout pregnancy, plateauing from 36 weeks of gestation and beyond [18,19].

Given that elevated serum bile acids down-regulated renal 11βHSD2 mRNA expression, we investigated 11βHSD2 gene expression in human and murine cholestatic placentas as well as in placental explants and choriocarcinoma cell lines cultured in medium containing different bile acids.

2. Methods

This study was approved by Hammersmith Hospital Research Ethics Committee (reference 97/5197), and all participants provided written informed consent.

2.1. Human placenta

Placental 11βHSD2 gene expression was analysed in placental samples taken from 10 women with ICP delivered at Queen Charlottes Hospital and compared to placental samples taken from 11 women with uncomplicated pregnancy over the same time. Women were diagnosed as having ICP if they presented with pruritus in association with liver dysfunction and serum bile acids ≥40 μmol/l. Exclusion criteria for ICP were other causes of hepatic dysfunction, including pre-eclampsia, the HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome, acute fatty liver of pregnancy, primary biliary cirrhosis, viral hepatitis and any ultrasound abnormality that may result in biliary obstruction. Exclusion criteria for control subjects were the same as those for cases, but also included a history of pruritus during the current or any previous pregnancy. Of the 10 women with ICP, 5 received treatment with UDCA and 5 were not treated. The clinical and demographic characteristics for pregnancies complicated by ICP are shown in Table 1. There was no significant difference in maternal age between cases 33.2 (±1.3) and controls 36.2 (±2.0) years. In keeping with obstetric practice at the study centre, the gestational week at delivery in the control group was higher compared to women with ICP 39.4 (±03) vs 37.4 (±0.1) ($p \leq 0.05$). However placental 11βHSD2 gene expression does not alter from 36 weeks gestation onwards [18,19]. Samples were not matched for mode of delivery as this has not been shown to influence placental 11βHSD2 mRNA expression [19–21]. Similarly, infant gender does not influence placental 11βHSD2 mRNA expression [22,23]. Samples from whole placenta were dissected and small segments (approximately 2 cm^3) were soaked in 5 times volume RNA *later*™ (Life Technologies Ltd, Paisley, UK) for 24 h, or snap frozen on dry ice within 30 min of delivery. Samples were subsequently stored at −80 °C until use.

2.2. Placental explants

Syncytial tissue was dissected from whole placenta under sterile conditions. Samples were placed into paired wells in 6 well plates containing netwell inserts (Corning). Each well contained 1.5 mls of RPMI–1640 media (including: 1% amphotericin, 1% penicillin/streptomycin and 10% fetal calf serum). Tissue was incubated under modified cell culture conditions (5% CO_2, 8% Oxygen and 37 °C) reflective of normal physiological conditions in the third trimester [24]. The media was changed on days 2 and 4. On day 5 the explants were washed in ice-cold PBS and exposed to differing concentrations and types of bile acids in phenol red free media for 24 h (as below).

2.3. Placental cell lines

Immortalised choriocarcinoma cells (Jeg, Jar, TCL1 and BeWo b30 clone) were routinely cultured using DMEM F12 media (Sigma–Aldrich, Gillingham, UK) containing: 1% amphotericin, 1% penicillin/streptomycin, 1% glutamine and 10% fetal calf serum under standard cell incubation conditions (5% CO_2, 21% Oxygen and 37 °C). Prior to confluence the cells were harvested using 1× Trypsin EDTA for 5 min after which it was inactivated with culture medium, centrifuged, re-suspended and then seeded at a concentration of 500,000 cells per well into 6 well plates. After 24 h the cells were washed in PBS solution, serum starved for 6 h and then differentiated with forskolin (20 μmol/l), in combination with differing concentrations (0–300 μmol/l) of unconjugated bile acids, (cholic acid (CA), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA)), or conjugated bile acids, (taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA) and tauroursodeoxycholic acid (TUDCA)) for 24 h in phenol red-free DMEM F12 medium (Sigma–Aldrich, Gillingham, UK); (including 1% amphotericin, 1% penicillin/streptomycin 1% glutamine and 2.5% charcoal stripped fetal calf serum). Samples were studied in triplicate.

2.4. Murine studies

Animal treatments were conducted in accordance with the Animals (Scientific Procedures) Act 1986 and the guidelines of The Centre of Biomedical Science, Imperial College, London. Procedures were approved by the Imperial College London ethical treatment of animals committee as previously described [25]. C57BL6 wild type mice were purchased from Harlan Laboratories (UK). Pre-conception, dietary supplementation with 0.5% cholic acid was assigned to one group (resulting in serum bile acids >100 μmol/), the other group was maintained on a standardised diet as previously described [25]. Humane killing was performed on the 18th day of gestation [25]. Placental tissue was then harvested, snap frozen in liquid nitrogen and stored at −80 °C.

2.5. Reverse transcription and Q PCR

Human and murine placental tissue was homogenised in 350 μl of beta-mercaptoethanol/RLT lysis buffer and RNA extracted using RNeasy mini kit™ columns (Qiagen, Crawley, UK) in accordance to the manufacturer's instructions. RNA Integrity Number values for human placentas ranged from 5.3 to 8.0. 1 ml of Trizol™ (Invitrogen–Life Technologies, Paisley, UK) was added to wells containing placental explants and choriocarcinoma cells and the RNA extracted in accordance with the manufacturer's instructions.

cDNA was synthesised as previously described [26]. Gene expression was studied in triplicate using 384 and 96 RT PCR well plates on the 7900 Real Time PCR system (Life Technologies). A total reaction volume of 8–25 μl, including 10–40 ng of cDNA respectively, was used in conjunction with Sybr green jump start™ (Sigma–Aldrich, Gillingham, UK). PCR analysis was performed under the following thermocycler conditions: 10 min 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Relative gene expression was calculated as $2^{-\Delta\Delta Ct}$, where ΔΔCt = ΔCt of the sample − ΔCt of the calibrator gene: Human 18S RNA (18S), L19 or Murine cyclophilin (mCyclo). In human placenta, quantification of syncytial tissue was made with reference to gene expression of beta human chorionic gonadotropin (β–hCG); a hormone whose expression is not affected by cholestasis [27] and relative 11βHSD2

Table 1
Maternal demographic data in ICP cases. Data expressed as mean values (±SEM). CTG = fetal cardiotocograph abnormality in (labour), EL CS = Elective Caesarean Section, ICP = Intrahepatic Cholestasis of Pregnancy, IOL = Induction of Labour, MEC = Meconium-stained amniotic fluid, NA = Not applicable, PNS = Post natal seizure, SROM = Spontaneous Rupture of Membranes, VD = Vaginal Delivery.

ICP	Gestation age (weeks)	Mode of delivery	SBA at delivery (μmol/l)	ALT at delivery (IU/)	Maternal age (years)	Adverse fetal outcome
1	37.0	(IOL) VD	120	370	34	MEC/CTG
2	37.7	(IOL) VD	43	85	30	MEC
3	38.1	(IOL) VD	96	491	34	–
4	37.3	El CS	54	65	32	–
5	36.9	El CS	100	496	36	MEC
6	37.4	(IOL) VD	103	284	29	–
7	38.0	(IOL) VD	56	41	36	–
8	36.9	(SROM) VD	92	24	31	PNS
9	37.3	(IOL) VD	101	187	31	–
10	36.9	El CS	69	532	36	–
Mean	37.4 (±0.1)	NA	83.4 (±8.2)	275.5 (±64.4)	33.2 (±1.3)	NA