Placenta 44 (2016) 91–97

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

The effect of 17α-ethynylestradiol induced intrahepatic cholestasis of pregnancy on placental P-glycoprotein in mice: Implications in the individualized transplacental digoxin treatment for fetal heart failure

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ARTICLE INFO

Article history:
Received 16 January 2016
Received in revised form
1 June 2016
Accepted 20 June 2016

Keywords:
Intrahepatic cholestasis of pregnancy
Placenta
P-glycoprotein
HIF-1α
Digoxin
Fetal heart failure

ABSTRACT

Introduction: Placental P-glycoprotein (P-gp) plays a significant role in controlling transplacental digoxin transfer rate. Investigations on P-gp regulation in placenta of women with different pregnant pathological states are of great significance to individualized transplacental digoxin treatment for fetal heart failure (FHF). This study aimed to explore the effect of 17α-ethynylestradiol induced intrahepatic cholestasis of pregnancy (ICP) on placental P-gp in mice.

Methods: ICP model in mice was induced by subcutaneous injection of 17α-ethynylestradiol dissolved in propylene glycol once daily from E12.5 to E16.5. Maternal plasma ALT, AST, TB, DBIL, γ-GT, LDH, ALP and TBA concentrations were measured. HE staining was applied for observation of maternal liver cells degeneration, necrosis and intrahepatic cholestasis. Placental *Abcb1a/Abcb1b/HIF-1α* mRNA and P-gp/HIF-1α protein expression were determined by real-time quantitative PCR and western-blot. Maternal plasma and fetal-unit digoxin concentrations were detected by a commercial kit assay.

Results: The ICP group showed higher levels of maternal plasma ALT, AST, TB, DBIL, γ-GT, LDH, ALP and TBA concentrations, reduction in fetal survival rates, lower placental and fetal weights, and typical liver cells degeneration, necrosis and intrahepatic cholestasis. The placental *Abcb1a* mRNA and P-gp expression of ICP group were significantly elevated, while transplacental digoxin transfer rates were significantly decreased. Both placental *HIF-1α* mRNA and protein expression was significantly elevated in the ICP group, and there was a positive correlation between *Abcb1a* mRNA and *HIF-1α* mRNA.

Conclusions: 17α-ethynylestradiol induced ICP could up-regulate placental P-gp expression and reduce transplacental digoxin transfer rate in mice, which might be partly associated with higher expression of *HIF-1α*.

1. Introduction

Congestive fetal heart failure (CFHF), defined as inability of the heart to deliver adequate blood flow to organs such as brain, liver, and kidneys, is a common final outcome of many intrauterine disease states that may result in fetal demise [1]. Over the past decades, the increasing rates of infertility and advances in fetal medicine have changed the attitude to CFHF from simply terminating the pregnancy by interruption to possible active therapy of

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http://dx.doi.org/10.1016/j.placenta.2016.06.008
0143-4004/

the fetus [2]. Nowadays, the treatment of CFHF usually consists of transplacental administration of digoxin as the drug of first choice [3]. Although transplacental passage of digoxin has been confirmed using technique of *ex vivo* perfused human term placenta, *in vivo* data have indicated relatively lower umbilical cord to maternal plasma drug concentration ratio with considerable inter-individual variability [4]. Our previous studies were consistent with these findings, showing that fetal-to-maternal digoxin concentration ratio at delivery ranges between 0.46 and 0.89, which might partially explain the variable inter-individual digoxin treatment effectiveness for CFHF [5,6]. Due to the aforementioned reasons and the narrow digoxin therapeutic window, individualized transplacental digoxin treatment for CFHF deserved to be quite essential and critical in clinic. Therefore, proper understanding of transplacental passage of digoxin and its influence factors will be helpful in guiding clinicians to more accurate and safer pharmacotherapy for CFHF.

In the past decades, several membrane transport proteins have been discovered in the placenta. There are evidences to support the role of those transporters in controlling the transplacental transfer of drugs [7]. Among them, the P-glycoprotein (P-gp) is most extensively studied currently. It is specially located in the maternal-facing apical membrane of placental syncytiotrophoblast and has the capacity to actively extrude a wide range of drugs back to the maternal circulation, thus decreasing drugs' transplacental transfer rates [8]. In humans, P-gp is encoded by *ABCB1* gene alone, whereas two closely located genes (*Abcb1a* and *Abcb1b*) independently encode the isoforms of this transporter in rodents. Digoxin has been widely proved to be P-gp substrate [9]. Studies in *Abcb1* knockout mice have shown that P-gp deficiency could result in many fold higher concentrations of digoxin in fetal compartment [10].

Several previous studies including ours have proved that maternal drug usage, maternal physiological and pathological factors could alter placental P-gp expression and thereby affect transplacental digoxin transfer rate [11–18]. These findings imply that the variable interindividual expression of placental P-gp could partially contribute to the variable transplacental digoxin transfer rate and treatment effectiveness for CFHF. Therefore, more investigations on P-gp expression and regulation in placenta of women with different physiological and pathological states are of great significance to the individualized transplacental digoxin treatment for CFHF.

The intrahepatic cholestasis of pregnancy (ICP) is the most common liver disease seen in pregnancy. The incidence of ICP varies widely among various locations and ethnicities (<1% in most populations), but affecting about 5% of pregnancies in China [19]. It is characterized by maternal pruritus, increased bile acids and liver transaminases in third trimester of pregnancy and is usually complicated by spontaneous preterm birth, fetal hypoxia and intrauterine fetal deaths [20]. Previous studies regarding ICP both in human and animals have indicated that the elevated bile acid in maternal blood might cause vasoconstriction of placental veins [21] and impair placental morphology and function [22], which could result in the occurrence of placental hypoxia [23]. Hypoxia in cancer cell lines was proved to be associated with increased P-gp expression and tumor resistance [24,25]. Additionally, recent studies have proposed that hypoxic conditions in first trimester placentas [15] and in placentas derived from preterm and pre-eclamptic pregnancies [13] are at least partially responsible for their relatively higher expression of placental P-gp. In light of these findings, it is conceivable that placental P-gp expression and transplacental digoxin transfer rate are mostly likely to be affected in pregnancies with ICP, which might be associated with placental hypoxia.

Currently, studies on placental P-gp expression and transplacental digoxin transfer rate in pregnancies with ICP are lacking. Experimental intrahepatic cholestasis induced by 17α-ethinylestradiol in rodents is a widely used *in vivo* model and has been validated in many studies [26–29]. Therefore, the present study was carried out to determine the effect of 17α-ethinylestradiol induced ICP on placental P-gp and transplacental digoxin transfer rate in mice, and to explore whether the hypoxia-inducible factor-1α (HIF-1α), an important and specific transcription factor for hypoxic adaption, was involved in regulation of placental P-gp or not.

2. Materials and methods

2.1. Animals

All female C57BL mice (8–10 weeks of age) used were purchased from Sichuan University Animal Institution. They were identically housed, maintained on a 12 h light/dark cycle and had access to rodent chow and water ad libitum. Pregnancy was defined after the presence of vaginal plug and designated as embryonic day [E] 0.5. All animal experiments were conducted in accordance with the National Institutes of Health Guide and with the approval of the Sichuan University Committee for the Care and Use of Laboratory Animals.

2.2. Establishment of 17α-ethynylestradiol induced ICP model in mice and sample collection

Experimental intrahepatic cholestasis induced by 17α-ethinylestradiol in rodents is a widely used *in vivo* model and has been validated in many studies [26–29]. Because 17α-ethinylestradiol is lipophilic, it needs to be dissolved in the propylene glycol as a vehicle. The final drug concentration of 17α-ethinylestradiol in dosing solution was 1.0 mg/ml. A total of 16 pregnant mice were randomly divided into the control group and the ICP group, with 8 animals in each group. In the control group, pregnant dams received propylene glycol (V900115-500 ml, sigma) by subcutaneous injection once daily from E12.5 to E16.5. In the ICP group, pregnant dams received 17α-ethynylestradiol (12,60,001–150 mg, sigma, 5 mg/kg) dissolved in propylene glycol by subcutaneous injection once daily from E12.5 to E16.5.

Digoxin was used as a pharmacological probe for assessing placental P-gp function both *in vivo* and *in vitro* studies as it is specifically transported by P-gp across the placenta and because of its biological stability. Therefore, after an overnight fast, digoxin (West China Second Hospital, Sichuan University) was administrated at a dose 50 μg/kg by gavages and dams were then euthanized 1 h later with chloral hydrate (West China Second Hospital, Sichuan University) at E17.5. The 1hr time point was chosen because our preliminary results demonstrated that Cmax of digoxin in maternal plasma was reached at 1 h post dose, while the concentrations in "fetal unit" are still high enough for detection at this time point. Maternal blood was collected via cardiac puncture. Plasma was separated and stored at −80 °C for analysis. Then, maternal abdomen was opened and a section of liver tissue taken from the same position was fixed in 4% formalin for hematoxylin-eosin (HE) staining.

To determine net transplacental transfer, "fetal-units" (2 per dam, randomly selected from both sides of the uterus) were collected and weighed at the time of dissection. The "fetal-unit" was comprised of all elements in direct contact with fetal placenta/labyrinth: the fetus, amniotic fluid and fetal membranes, but not the placenta. Considering that the amniotic fluid and fetal membranes contain substrate which was traversed the placenta from