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The effect of maternal obesity on the expression and functionality of placental P-glycoprotein: Implications in the individualized transplacental digoxin treatment for fetal heart failure

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ABSTRACT

Introductions: Placental P-glycoprotein (P-gp) plays a significant role in controlling digoxin transplacental rate. Investigations on P-gp regulation in placenta of women with different pregnant pathology are of great significance to the individualized transplacental digoxin treatment for fetal heart failure (FHF). This study aimed to explore the effect of maternal obesity on the expression and functionality of placental P-gp both in human and in mice.

Methods: Placenta tissues from obese and lean women were collected. Female C57BL mice were fed with either a normal chow diet or a high-fat diet for 12 weeks before mating and throughout pregnancy. Maternal plasma glucose, HDL-C, LDL-C, TC, TGs, insulin, IL-1β, IL-6 and TNF-α concentrations was detected. Placental *ABCB1/Abcb1a/Abcb1b/IL-1β/IL-6/TNF-α* mRNA and P-gp/IL-1β/IL-6/TNF-α protein expression were determined by real-time quantitative PCR and western-blot, respectively. Maternal plasma and fetal-unit digoxin concentrations were detected by a commercial kit assay.

Results: Both *ABCB1* gene mRNA and protein expression of obesity group was significantly lower than that of control group in human. The high-fat dietary intervention resulted in an overweight phenotype, a significant increased Lee's index, higher levels of plasma glucose, HDL-C, LDL-C, insulin and TGs, increased peri-renal and peri-reproductive gland adipose tissue weight, and larger size of adipose cell. Compared with control group at the same gestational day (E12.5, E15.5, E17.5), placental *Abcb1a* mRNA and P-gp expression of obese group were significantly decreased in mice, while digoxin transplacental rates were significantly increased. Higher maternal plasma IL-1β/TNF-α concentrations and placental IL-1β/TNF-α expression were observed in obesity groups in comparison with control group at the same gestational age.

Conclusions: Maternal obesity could inhibit placental P-gp expression and its functionality both in human and in mice, which might be resulted from a heightened inflammatory response.

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 lead to fetal demise [1]. Over the past several decades, the increasing rates of infertility and advances in

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fetal medicine have changed the attitude to CFHF from simply terminating the pregnancy by interruption to possible active therapy of the fetus [2]. Nowadays, the treatment of CFHF usually consists of the 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 treatment effectiveness [5,6]. Due to the aforementioned reasons and the narrow digoxin therapeutic window, individualized transplacental digoxin treatment for fetal heart failure deserved to be quite essential and critical in clinic. Therefore, proper understanding of transplacental passage of drugs and its influence factors will be helpful in guiding the clinicians to more accurate and safer pharmacotherapy during pregnancy.

There is growing evidences that passage of drugs across the placenta can no longer be determined simply by their own physical–chemical properties or the effect of hemodynamic changes in the maternal compartment during pregnancy. Several drug transporters have been discovered in the placenta and are widely proved to have a significant role in drug transfer across the placental barrier [7]. Among them, the P-glycoprotein (P-gp) is most extensively studied currently. It is specially located in the maternal-facing apical membrane of the syncytiotrophoblast of placenta and has the capacity to actively extrude a wide range of drugs back to the maternal circulation, thus decreasing drugs' transplacental rates [8]. In humans, only one gene (*ABCB1*) is required to encode P-gp, whist two genes (*Abcb1a* and *Abcb1b*) are necessary to encode this transporter in rodents. Digoxin has been widely proved to be a substrate of P-gp [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 studies and our previous studies have indicated that placental P-gp could be regulated by maternal drug usage, maternal physiological and pathological factors, and thereby influence the digoxin transplacental rate [11–17]. These findings imply that the variable interindividual expression of placental P-gp could partially contribute to the variable digoxin transplacental rate and treatment effectiveness. Thus, more investigations on P-gp expression and regulation in placenta of women with different physiology and pregnant pathology are of great significance to the individualized transplacental digoxin treatment for fetal heart failure.

Maternal obesity was a common condition during their childbearing years worldwide [18–20]. Obesity was always manifested with several metabolic disorders such as insulin insensitivity, hyperglycemia and dyslipidemia, which could lead to inflammatory activation. Inflammation is a symptom of many pregnancy-related pathologies including infections, preeclampsia and gestational diabetes mellitus (GDM). Previous studies have proved that placental P-gp could be significantly altered in women with preterm labor with inflammation [13], or in rat dams with streptozotocin-induced GDM [21]. Additionally, many pro-inflammatory cytokines mainly including IL-1β, IL-6 and TNF-α, used as the ubiquitous and surrogate markers of inflammation, could cause a down-regulation of P-gp in many cell types [22–24].

Currently, studies on placental P-gp expression and functionality in pregnancies with maternal obesity are lacking. Additionally, findings of altered P-gp expression by several pro-inflammatory cytokines and in pathological pregnancies mentioned above offer some clues that may be applicable to maternal obesity. In light of these findings, we hypothesized that placenta from maternal obesity could be exposed to higher levels of inflammatory cytokines, either from the mother or from the placenta itself, which might lead to the down-regulation of placental P-gp and consequently compromise placental barrier function. In the present study, a high-fat diet induced maternal obesity model of mice was established and human placentas from obese women were collected to determine the effect of maternal obesity on the placental P-gp expression and its functionality.

2. Materials and methods

2.1. Human placenta collection

Placentas from 20 lean and 10 obese women were consecutively obtained from mothers, who delivered at the Department of Gynecology and Obstetrics of our hospital by a well-trained nurse. Obesity was defined as pre-gravid body mass index (BMI) $\geq$28 kg/m2 in accordance with the criteria specially formulated for Chinese [25]. Only placentas from healthy Han Chinese mothers with uncomplicated pregnancy and neonates with a gestational age between 38 and 41 weeks and a birth weight between 2500 and 4000 g were included. Those samples from mothers with chronic diseases (eg. Hepatitis, hypothyroidism, polycystic ovary syndrome, and sexually transmitted infections), pregnant complications (eg. gestational diabetes mellitus, pregnancy-induced hypertension and intrahepatic cholestasis of pregnancy), and any medications use were excluded.

Immediately after delivery, large tissue cores through the full thickness of the placenta were obtained in each quadrant, avoiding chorionic plate tissue and areas with obvious evidence of thrombosis or other abnormalities. The tissues were immediately frozen in liquid nitrogen and stored at −80 °C until use. In addition, the maternal and neonatal clinical data were also collected, including maternal age, maternal ethnicity, maternal weight, maternal height, health conditions, pregnancy complications, any medication consumptions, and neonatal birth outcomes (eg. sex, gestational duration, birth length, birth weight and placental weight).

Informed written consent was obtained from those mothers when we collected the placental tissue. The study was performed according to the principles of Good Clinical Practice and the Declaration of Helsinki and approved by the University Committee on Human Subjects at Sichuan University.

2.2. Establishment of high-fat diet induced maternal obesity model in mice and sample collection

All animals were purchased from Sichuan University Animal Institution and identically housed, maintained on a 12 h light/dark cycle and had access to rodent chow and water ad libitum. 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.

In total, ninety female C57BL mice (6 weeks) were randomly divided into the control group (n = 40)and the obesity group (n = 50). They were fed with the basal diet (4.4% fat) and the high-fat diet (15% fat), respectively. Both diets were purchased from DaShuo Biotechnology Co., Ltd (Chengdu, Sichuan, China). Body weights and length of all experimental mice were measured weekly. The following criteria were used to confirm the successful establishment of this high-fat diet-induced obesity model: 1) the average weight of the obesity group was increased by over 20% compared with the control group; 2) the Lee's index [defined as $\sqrt[3]{weight(g)*1000/length\ (cm)}$] [26] of the obesity group was significantly higher than that of the control group. After 12 weeks dietary intervention, the mice from two groups were made to mate