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Review

Placental transfer and metabolism: An overview of the experimental models utilizing human placental tissue

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ABSTRACT

Over the decades several *ex vivo* and *in vitro* models which utilize delivered human placenta have been developed to study various placental functions. The use of models originating from human placenta to study transplacental transfer and related mechanisms is an attractive option because human placenta is relatively easily available for experimental studies. After delivery placenta has served its purpose and is usually disposed of. The purpose of this review is to give an overview of the use of human placental models for the studies on human placental transfer and related mechanisms such as transporter functions and xenobiotic metabolism. Human placental perfusion, the most commonly used continuous cell lines, primary cells and tissue culture, as well as subcellular fractions are briefly introduced and their major advantages and disadvantages are discussed.

Contents

1. Introduction

Human placenta plays an important role in the development of the fetus, being the major interface between mother and fetus. Placenta provides a gateway to oxygen and nutrients from the mother to the fetus, produces hormones to support the pregnancy and serves as an excretion pathway for various metabolites and carbon dioxide. However, it also plays a role in the exposure of the fetus to potentially toxic xenobiotics via the maternal circulation. Transport processes across the placenta and metabolism in the placenta may determine the exposure of the fetus to xenobiotics. Therefore, the studies of these processes are important when trying to predict possible fetal exposure. Our review describes different models that are used to study transplacental transfer and related processes, and discusses their major advantages and disadvantages.

Human placenta starts to develop during early pregnancy. Even before the placenta is formed, a few days after fertilization, the first specialized placental cells, trophoblasts, are present forming the outer layer of the blastocyst. Thus, already at this stage barrier of trophoblastic cells may protect the embryo from xenobiotics. During implantation blastocystic trophoblasts attach to and invade into the uterine lining. The trophoblast cells of the implanting embryonic pole differentiate into two trophoblastic layers: a layer of mononuclear cytotrophoblasts and the multinucleated syncytiotrophoblast. These structures will develop into the placenta during

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the first trimester. The villous structure of the human placenta is gradually formed already by 18–20 days after fertilization (Benirschke et al., 2006). However, during the first trimester the cytotrophoblastic plugs partially occlude the lumen of spiral arteries reducing the amount of maternal blood entering the intervillous space. Using the 3-dimensional vaginal ultrasound and power Doppler angiography, maternal intervillous blood flow has been detected from 6th gestational week onwards with a gradual increase during the first trimester (Mercé et al., 2009).

From a toxicological point of view it is important that the placenta provides only meager protection for the fetus against various xenobiotics. The mechanisms for transplacental transfer include passive diffusion, active transport, facilitated diffusion, filtration and pinocytosis. Passive diffusion is an important mechanism for transplacental transfer of xenobiotics. Physicochemical properties of xenobiotics such as molecular weight, pKa and lipid solubility affect the transplacental transfer by passive diffusion. Very large chemical compounds with a molecular weight of over 500D are generally transferred incompletely across the placenta (for a review see e.g. Myllynen et al., 2007). Both transporter proteins and xenobiotic metabolism enzymes have been found in human placental tissue, and their significance in transplacental transfer is being actively studied.

Human placenta expresses a number of transporter proteins. In syncytiotrophoblast, various transporters have been found both in the brush-border (apical membrane) facing maternal blood and basolateral membrane close to fetal capillaries (for extensive reviews see e.g. Vähäkangas and Myllynen, 2009; Ni and Mao, 2011). Depending on the localization and function, transporters may either increase or decrease xenobiotic transfer through the placenta towards fetal circulation. The expression of the transporters found in the apical and basal membranes of the syncytiotrophoblast differs leading to polarized transport across the structure. Complexity is further increased by the fact that also cells in fetal capillaries harbor transporters. Multiple studies in several species including human suggest that several ABC (ATP binding cassette) transporters decrease fetal exposure to xenobiotics (see e.g. Lankas et al., 1998; Kalabis et al., 2007; Pávek et al., 2001; Smit et al., 1999; Mölsa et al., 2005; Myllynen et al., 2008). In mouse, the inhibition of the ABCB1 transporter (p-glycoprotein, MDR1) has been reported to increase teratogenicity of ivermectin (Lankas et al., 1998). It is important to get more insight in the role of human placental transporters in fetal exposure because the mechanisms of transplacental transfer of many chemicals are insufficiently known.

Placental metabolism may also affect fetal exposure to xenobiotics. Compared to the liver the activities of xenobiotic metabolizing enzymes in human placenta are generally low (Hakkola et al., 1998). Still, placental metabolism may lead to metabolites with toxic potential compared to the parent compound. For instance, human placenta can metabolize retinoids (isotretinoin and tretinoin) to both more and less toxic metabolites (see e.g. Miller et al., 1993) and benzo(a)pyrene to active metabolites binding to DNA (e.g. Karttunen et al., 2010). The expression of xenobiotic metabolizing enzymes is dependent on the developmental stage of the placenta (Myllynen et al., 2007). Quite a few CYP (cytochrome P450) enzymes are expressed at the mRNA level, especially during the first trimester (Hakkola et al., 1998; Myllynen et al., 2007). However, of the CYP enzymes from the groups 1–3, only CYP1A1 seems to be functional in human placenta. In term placenta the CYPs 4B1 and 19 (steroid aromatase) are active and contribute to the metabolism of some xenobiotics, e.g. aflatoxin B1 (Storvik et al., 2011; Hakkola et al., 1998). One of the most recently described CYPs, CYP2S1, is also expressed in the placenta and has both endogenous and xenobiotic substrates (Bebenek et al., 2012). Its role in placental toxicology remains to be studied. At least the following metabolizing enzyme activities have been described in term human placenta: nitric oxide synthase, quinine reductase, nitroquinoline oxidase and alcohol dehydrogenase). Of the transferases, activities of glutathione-S-transferase, glucuronyltransferase as well as sulfotransferase are found from the first trimester on, and *N*-acetyltransferase at least in term placenta (for a review see e.g. Myllynen et al., 2007).

Currently, for ethical and practical reasons animal experiments and testing are being replaced with *in vitro* models whenever possible. To predict exposure of human fetus to chemicals, several relevant *in vitro* models for the study of human placental transfer including transporter function and metabolism have been developed over the past decades. The purpose of this review is to give an overview of such models and to compare their usefulness for estimating human placental transfer and metabolism.

2. *Ex vivo* human placental perfusion

Human placental perfusion of a single cotyledon with separate maternal and fetal circulations was originally introduced by Panigel (1962) and further developed by the groups of Schneider et al. (1972) and Miller et al., (1989), (Table 1). Placental perfusion is the only experimental model to study human transplacental transfer in organized human placental tissue. So far there are no standardized criteria for *ex vivo* human placental perfusion. Placentas from both vaginal births and C-sections can be used for the perfusion. We reported recently that the method of delivery does not affect the transplacental transfer of antipyrine, ethanol or two food borne carcinogens, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (Mose et al., 2012).

Ex vivo placental perfusion may be either nonrecirculating (open/single-pass) or recirculating (closed) perfusion. The methodological details, such as composition of maternal and fetal perfusates, flow rates in maternal and fetal circulations, composition of gas mixtures used to oxygenate the tissue and perfusate volumes all vary from one laboratory to another. In most published studies human placental perfusions have been carried out for 2–6 h although it is possible to perfuse placentas for extended periods of time, up to 48 h (Polliotti et al., 1996; Miller et al., 1989; Heikkilä et al., 2002; Woo et al., 2012).

Various markers are used to monitor placental viability during the perfusion and the selection of markers may vary between research groups (see e.g. Myllynen et al., 2010; Vähäkangas et al., 2011). Minimal loss of fetal perfusate is the main marker of a successful perfusion. Volume loss of 2–3 ml from fetal circulation is usually considered acceptable. Recently, a loss greater than 3 ml/h has been associated with higher transfer of the control substance FITC-dextran which in successful perfusions does not cross placental membranes due to its high molecular weight (Mathiesen et al., 2010). Antipyrine or other reference compounds such as creatinine are used to confirm the overlap between maternal and fetal circulations. These markers may also be used to normalize the perfusion data for interindividual variation. Other markers used for tissue viability include pH, oxygen consumption, net oxygen transfer, glucose consumption, lactate production and tissue morphology after perfusion. Hormone production (human chorionic gonadotropin or hCG, human placental lactogen or hPL, leptin) is also a good marker of functional placental tissue.

Although placentas from 27–40 weeks have been perfused (Fokina et al., 2011; Nanovskaya et al., 2008), the challenging perfusion method is usually applied to term placenta. Even then the success rate, counting from the placentas good enough to be hooked to the perfusion equipment, is only about 50%. Thus, the major limitation of this technique is that it does not provide data