New Aspects in Placental Drug Transfer

Gershon Holcberg MD[1], Marina Tsadkin-Tamir MSc[1,3], Olga Sapir MD[1], Mahmoud Huleihel PhD[2], Moshe Mazor MD[1] and Zvi Ben Zvi PhD[3]

Departments of [1]Obstetrics and Gynecology, [2]Microbiology and Immunology, and [3]Clinical Pharmacology, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

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Abstract

The human placenta is the interface between the mother and fetus in the uterus. Until recently it was generally believed that the uterus provides a protective environment for the fetus. It is now accepted that any chemical substance, including any therapeutic agent, administered to a mother is able to permeate across the placental barrier. Unfortunately, the placental transfer of substances and their distribution in the placenta is not well established. Understanding the structure of placental transporters and their function may serve as the ideal tool for drug development and the cure of mother and fetus during pregnancy.

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Until the appearance of the thalidomide-induced birth defects in the early 1960s, it was generally believed that the uterus provides a protective environment for the fetus. For the last four decades, the liability to control drug distribution across the placenta has been accepted; however, improved safety and selectivity of drug therapy in pregnancy has not necessarily been a priority in drug design and development.

It is clear that any drug or chemical substance administered to the mother is able to cross the placenta to some extent unless it is metabolized or altered during passage or its molecular size and low lipid solubility pass the limit for a transplacental transfer [1]. Several studies indicate that human fetuses are widely exposed to prescription and non-prescription drugs prenatally or during labor and delivery [2]. Nonetheless, the placenta is still an effective barrier for the developing fetus, preventing the entry of various xenobiotics from the mother to the fetus and facilitating the passage of various xenobiotics from the fetus to the mother [3]. Placental transport from the fetus to the mother is established only in the fifth week of fetal life. Several transport systems that recognize a wide variety of pharmacologic active drugs as substrates have been identified in the placenta [3].

Traditionally, teratogenic effects of drugs have been noted as anatomic malformations that are time and dose-dependent. The fetus is at greater risk during the first 3 months of gestation – organogenesis. However, it is possible for drugs and chemicals to exert their effects on the fetus later in pregnancy. The mechanisms whereby drugs exert teratogenic effects are poorly understood, particularly in humans [1]. The most important therapeutic strategy is to reduce unnecessary fetal exposure to drugs required by the mother or, in some instances, to reduce maternal exposure to drugs destined for fetal treatment [4].

Transplacental transfer

The human placenta is a unique organ whose structure is different to that of other animal species. Unlike other species, the human placental barrier comprises a single rate-limiting layer of multinucleated cells called syncytiotrophoblasts. During placentation in the human, fetal tissues erode the maternal blood vessels to attain a closer proximity to the maternal surface of the villi, which are covered by a layer of syncytiotrophoblasts with interspersed precursor cells – the cytotrophoblasts [4]. Underlying the syncytiotrophoblast is a basement membrane shared with endothelial cells of the fetal capillary; however, the rate-limiting barrier for permeation across the human placenta is the syncytiotrophoblast layer [4]. One of the major functions of the placenta is to mediate the transfer of nutrients from the mother to the fetus and to eliminate metabolic waste products from the fetus.

Drugs given to mothers have the potential to cross the placenta and reach the fetus. In certain circumstances, a comparison of the drug concentration in the maternal and fetal plasma reflects the exposure of the fetus to the maternally administered drugs. Several drugs rapidly cross the placenta and pharmacologically significant concentrations equilibrate in maternal and fetal plasma. Their transfer is termed "complete." Other drugs cross the placenta incompletely, and their concentrations are lower in fetal than in maternal plasma. A limited number of drugs reach greater concentrations in fetal than in maternal plasma, the so-called exceeding transfer. The following parameters were considered possible factors determining the extent of placental transfer: the molecular weight of the drug, the pKa (pH at which the drug is 50% ionized), and the extent to which the drug binds to the plasma protein. Drugs with molecular weights greater than 500 D have an incomplete transfer across the human placenta. Strongly dissociated acid drug molecules should have an incomplete transfer, but this does not seem to be an absolute rule [5]. Moreover, several multispecific transporters are involved in the transport of drugs and xenobiotics.

Placental transporters

The placenta contains a number of transporters, some of which appear to be specifically dedicated to removal of xenobiotics and

toxic endogenous compounds. Active drug efflux transporters of the ATP binding cassette-containing family of proteins have a major impact on the pharmacologic behavior of most drugs used today. Pharmacologic properties affected by ABC transporters include oral bioavailability, as well as hepatobiliary, direct intestinal, and urinary excretion of drugs and drug metabolites and conjugates. ABC transporters can extensively limit the penetration of drugs into pharmacologic sanctuaries, such as brain, testis and fetus. The interaction with ABC transporters determines the clinical usefulness, side effects and toxicity risks of drugs [6]. It is generally accepted that the physiologic function of the ABC transporters is to provide protection from a diverse group of xenobiotics. Moreover, they may protect tissues from the harmful effects of endogenous compounds (steroids).

Drug efflux transporters – such as P-glycoprotein, several multidrug-resistant associated proteins, and breast cancer-resistant protein – provide mechanisms that protect the developing fetus. However, to date, there are only eight known MRPs, six of which have been fully sequenced [7].

Recently St-Pierre and co-workers [8] described a family of MRPs in human placenta. Human placenta at term expresses at least three members of the MRP family: MRP1, MRP2 and MRP3. The immunoreactivity to MRP1 in term placenta was observed mainly in the fetal blood vessels of the terminal and intermediate villi; MRP1 was localized to the endothelium of the vessels. MRP2 was detected in the apical membranes of the syncytiotrophoblast and not in fetal blood vessels. Its expression profile therefore differs from that of MRP1 and resembles that of Pgp, which is also absent in endothelium but present in apical membranes. MRP3 was detected in the syncytiotrophoblast. The physiologic function of MRP-related proteins in the syncytiotrophoblast layer remains speculative. It is logical to expect that the apical trophoblastic expression of an ATP-dependent pump will be capable of transporting organic anions such as sulfate, glucuronide and glutathione conjugates out of the cell in order to protect the human fetus [8].

P-glycoprotein

Pgp, a 170 kDa protein, belongs to the family of plasma membrane proteins encoded by the *MDR* (multidrug resistance) genes that are well conserved in nature. *MDR* belongs to the superfamily of ABC transporters.

The molecular architecture of *MDR* shows a four-domain arrangement, with two transmembrane domains and two nucleotide-binding domains. Several mutational analytical approaches have been used to elucidate the mechanism of action of human Pgp. They indicated that mutations in the transmembrane domain or the nucleotide-binding domain are involved in the binding and transport of Pgp [9]. Subsequent genetic and biochemical data are consistent with the organization of Pgp, which can be viewed as two half molecules, each consisting of one integral transmembrane domain and one nucleotide-binding domain. The amino acid sequences of the two half molecules are closely related to each other, suggesting a pseudo-symmetry to the protein structure.

Mode of action

The majority of published data suggests that Pgp acts as a transmembrane pump that removes drugs from the cell membrane and cytoplasm. ATP hydrolysis provides the energy for active drug transport that can occur against steep concentration gradients. It has been proposed that Pgp acts like a hydrophobic vacuum cleaner or flippase. Pgp intercepts the drug as it moves through the lipid membrane and flips the drug from the inner leaflet of the plasma membrane lipid bi-layer to the outer leaflet and into the extracellular medium [9].

Pgp substrates

Chemicals transported by Pgp have very diverse structures that share only the properties of being hydrophobic amphipathic molecules (molecules having two sides with characteristically different properties). They include anti-cancer drugs such as doxorubicin, vinblastine, vincristine, etoposide and paclitaxel; immunosuppressive drugs such as cyclosporine A; steroids like hydrocortisone, cortisol, corticosterone and dexamethsone; anti-human immunodeficiency virus drugs such as ritonavir and saquinavir; cardiac drugs such as digoxin and quinidine; the lipid-lowering agent lovastatin; and the fluorescent dye rhodamine-123. As an exporter, Pgp activity decreases the intracellular concentration of Pgp substrates [10–12].

Pgp inhibitors include the immunosuppressant cyclosporin A and its non-immunosuppressive analogue PSC833 (valspodar); the calcium channel blocker verapamil; progesterone and the progesterone antagonist mifepristone (RU 486); the anti-arrhythmic agent quinidine; the anti-estrogen tamoxifen; the antibiotic erythromycin; the antifungal ketoconazole; and the cyclopropyldibenzosuberane LY 335979 [10,12–14].

Hoffmeyer et al. [15] identified a polymorphism in the human *MDR1* gene and described its distribution in a Caucasian population. The discovery and characterization of variation in the *MDR1* gene and diagnostic tests for the discrimination of different *MDR1* alleles in human individuals may provide a potent tool for improving the therapy of diseases with drugs that are substrates of Pgp

Early studies suggest that the *MDR* gene is expressed in human placenta and that this expression increases dramatically during pregnancy. Some of the cytoplasmic Pgp is seen in the placental cells during the first trimester. However, in the full-term placenta Pgp was detected only in the Hofbauer cells, suggesting synthesis at this developmental stage [16]. Several *in vitro* studies showed that Pgp activity is inhibited by progesterone [17]. Ushigome et al. [18] demonstrated that the uptake of digoxin, vincristine and vinblastine is significantly enhanced in the presence of progesterone. At term, progesterone levels range from 100 to 200 ng/ml plasma, and the placenta produces about 250 mg progesterone per day [19]. It is conceivable that the high levels of progesterone in the term placenta completely inhibit the activity of Pgp.

ABC = ATP binding cassette
MRP = multidrug-resistant associated protein
Pgp = P-glycoprotein

Both quinidine and verapamil inhibit the transporting function of Pgp in renal tissue [20]. We recently showed that Pgp inhibitors like quinidine and verapamil did not affect the transfer of digoxin in perfused human placenta *in vitro*. We propose that Pgp activity in placenta could be dependent on placental age and is related to placental maturation as well as to disruption in continuity of the trophoblast and redistribution of Hofbauer cells in the placenta. The high progesterone levels in the human placenta, as also observed in our experiments, may inhibit Pgp activity and drug transfer through the placenta from the mother to the fetus [21].

Hypoglycemic drugs and placental transfer

Hyperglycemia is associated with adverse outcome of pregnancy in women with gestational or preexisting diabetes mellitus. When diet and exercise alone prove inadequate, the standard treatment to control blood glucose levels in both gestational and pre-gestational diabetes has been insulin.

Due to its high molecular weight insulin is not able to cross the placenta. Insulin pharmacodynamics are altered during pregnancy, as evidenced by significantly lower insulin-mediated glucose disposal during pregnancy with increased insulin resistance. However, insulin pharmacokinetics are not significantly altered by pregnancy, since the volume of distribution, insulin clearance rate and half-life during the pregnancy remain virtually the same [22]. Whereas early *in vitro* studies suggested that insulin does not cross the human placenta [23], a subsequent placental perfusion study demonstrated that a small amount of human insulin, representing 1–5% of the insulin concentration in the maternal artery, was transferred from the maternal to the fetal circulation [24]. Moreover, even insulin does not normally cross the placenta on its own, but does so as insulin-antibody complexes. The amount of transfer is directly correlated with the amount of anti-insulin antibodies in the mother [25].

The use of human insulin minimizes the production of anti-insulin antibodies. However, these antibodies do cross the placenta because they could be measured in cord blood in concentrations proportional to those in the maternal blood [26]. It is not known whether the presence of these antibodies promotes transplacental transport of insulin and no correlation was found in the passage of antibodies [25].

A fast-acting form of insulin, lispro insulin, consists of two amino acids in the human insulin molecule, proline and lysine, which were reversed to create insulin lispro. This results in a weaker tendency for self-association after subcutaneous injection, more rapid absorption of the insulin, faster onset, and shorter duration of action as compared to regular insulin [27]. The new insulin was tested in pregnant diabetic women and showed fewer hypoglycemic episodes and similar levels of metabolic control as regular insulin. Anti-insulin antibody levels were similar to regular insulin, and fetal or neonatal abnormalities were not observed [28].

The use of insulin lispro in type 1 diabetes during pregnancy results in outcomes comparable to those in other large studies of diabetic pregnancy [29]. Lack of transfer of insulin lispro across the placenta was suggested in a study of the drug in women with gestational diabetes [30]. In that study no insulin lispro was detected in the umbilical cord blood of the infants when women received an intravenous infusion of insulin lispro during labor. In the *in vitro* study, maternal insulin lispro led to a small transfer of insulin lispro across the placenta when very high concentrations of insulin lispro were used. It seems that insulin lispro is safe in pregnancies complicated by diabetes. However, while insulin therapy is effective in achieving the appropriate levels of glycemia, it is inconvenient and expensive. An alternative approach would be attractive [31].

Sulfonylureas have been implicated as teratogens in animals and humans. A recent meta-analysis of all available data to date failed to show an increased teratogenic risk of sulfonylurea drugs during pregnancy [32]. Several reports point to a moderate to high placental transfer and slow elimination of the first-generation sulfonylureas (tolbutamide and chlorpropamide) from the neonatal circulation [33,34]. Clinical trials on newer generation sulfonylurea drugs (glibenclamide, glipizide, giclazide) found these drugs to be equally effective as insulin in achieving good glybetic control and perinatal outcome [35].

In vitro studies showed that gliburide only minimally crosses the placenta in a single human cotyledon perfusion. The reasons for poor placental transfer of gliburide may be its very high protein binding (99.8%) and relatively short elimination half-time (4–9 hours). Gliburide has been shown to both inhibit and to be a substrate to P-glycoprotein, which could potentially lead to many different drug interactions in both mother and fetus, especially in complicated pregnancies where the mother is taking several concomitant medications.

There is a paucity of data on the use of metformin in pregnant women. Only a partial barrier to metformin was observed, based on fetal drug concentrations. Furthermore, no *in vitro* placental transfer studies on metformin have been reported. Hellmuth et al. [37] noted that in women who were diabetic before conception, treatment with metformin during pregnancy was associated with an increased prevalence of preeclampsia and higher perinatal mortality compared to women taking sulfonylurea or insulin. However, some data [38,39] on women with polycystic ovarian syndrome and on diabetic women and non-diabetic women with gestational diabetes point to the safety and pregnancy-enhancing efficacy of metformin. Moreover, in PCOS, the use of metformin is associated with a tenfold reduction in gestational diabetes and a reduction of insulin resistance and insulin secretion, thus decreasing the secretory demands imposed on pancreatic B cells by insulin resistance and pregnancy [38].

Oral agents are the mainstay of pharmacologic treatment for non-pregnant women with type 2 diabetes. Because of the similarity between gestational and type 2 diabetes, some of these drugs could be used effectively and safely as alternatives to insulin therapy.

Conclusions and future trends

Evidence that most of the drugs administered to the mother are transferred transplacentally has changed the old concept of the invulnerability of the fetus due to the protection afforded by the

PCOS = polycystic ovarian syndrome

placental barrier. The commonly used drugs act on the fetus by means of two different mechanisms: directly after the placental transfer, and indirectly influencing the utero-placental circulation and maternal homeostasis. Fetal effects can be manifold, depending on the type of the drug, the dosage, the route of administration, gestational age in relation to the fetal ontogenesis, and the amount of drug that reaches the fetus

The placenta expresses several transporters that are relevant to drug distribution across the maternal-fetal interface. Most of these transporters perform vital physiologic functions in facilitating the transfer of nutrients and other normal metabolites across the placenta, but many of them also recognize xenobiotics and drugs as substrates due to structural resemblance to the physiologic substrates. With increasing knowledge on the substrate specificity of various placental transporters, it is evident that the placenta is not an effective barrier in protecting the developing fetus against harmful drugs. Although some transporters do function in preventing the entry of xenobiotics and drugs into the fetoplacental unit, several transporters actually facilitate the entry of xenobiotics. A thorough understanding of the role of various transporters in the placenta in the handling of drugs across the maternal-fetal interface is essential to evaluate the pharmacologic and toxicologic potential of therapeutic agents and drugs used by the mother during pregnancy.

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Correspondence: Dr. G. Holcberg, Dept. of Obstetrics and Gynecology, Soroka University Medical Center, P.O. Box 151, Beer Sheva 84101, Israel.
Phone: (972-8) 640-0360
Fax: (972-8) 627-5338
email: holcberg@bgumail.bgu.ac.il