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Abstract:

A challenge in modern drug therapy is to develop strategies for safer and more selective targeting of drug delivery in pregnancy. Specifically, approaches are needed that would restrict unnecessary drug exposure to either mother or fetus. There is evidence emerging that indicates the placenta does express natural transport and metabolism processes that function to control drug and nutrient distribution between the mother and fetus. Further, *in vitro* techniques developed in the past ten years now provide some of the tools necessary to elucidate transport and metabolism processes typical of the human placenta. As a consequence, pharmaceutical scientists are in a position to contribute significantly to the design and development of drugs for pregnancy.

Text of paper:

CONTROLLING DRUG DELIVERY ACROSS THE PLACENTA: A COMMENTARY

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1.   Introduction

Until the thalidomide-induced birth defects occurred in the early 1960's, physicians generally believed that the uterus provided a protective environment for the fetus. Subsequently, it has become accepted that any chemical substance, including any therapeutic agent, administered to a mother is able to permeate across the placental barrier (Yaffe, 1998).   For the last four decades, the inability to control drug distribution across the placenta has been accepted and progress in establishing improved safety and selectivity of drug therapy in pregnancy has not necessarily been a priority in drug design and development.   There is a continuing need for many mothers to continue to receive medications for epilepsy, diabetes, asthma, and a variety of other medical conditions during pregnancy. In general, drug use in pregnancy is on the rise over recent years (Yaffe, 1998). This has been attributed to a lack of awareness of the consequences of drug and chemical exposure. Consistent with that observation, and also of great concern, is the finding that drug therapy in pregnancy is now often without a specific rationale other than to treat the symptoms accompanying pregnancy (Yaffe, 1998).

Comment is provided here to indicate that there is evidence suggestive of the placenta's ability to control fetal exposure to drugs. Experimental tools have also been developed in the past decade to investigate fundamental placental mechanisms.   Pharmaceutical scientists have the opportunity to employ these *in vitro* techniques to investigate human placental transport and metabolism mechanisms and play a leadership role in devising strategies to better control drug delivery in pregnancy to both mother and fetus.

2.   The human placental barrier

Unlike other species (Enders and Blankenship, 1999), the human placental barrier is comprised of a single rate limiting layer of multinucleated cells, the syncytiotrophoblasts.   During placentation in

the human, fetal tissues erode the maternal blood vessels to attain a closer proximity to the maternal circulation. Chorionic villi containing fetal blood vessels penetrate into the maternal vessels and establish a sinusoid in which the villi are bathed by maternal blood (Dancis, 1987; Enders and Blankenship, 1999). At the cellular level, the maternal surface of the villi is covered by a layer of syncytiotrophoblasts with interspersed precursor cells called cytotrophoblasts or Langhans cells. Several cytotrophoblasts fuse or aggregate to form a multinucleated syncytiotrophoblast (Ringler and Strauss, 1990). Underlying the syncytiotrophoblast is a basement membrane shared with the endothelial cells of the fetal capillary, however, the rate-limiting barrier for permeation across the human placenta is the syncytiotrophoblast layer (Ala-Kokko et al., 1993; Sibley, 1994; Enders and Blankenship, 1999).

The predominant mechanism by which substances cross the human placental barrier is simple passive diffusion either transcellularly through the plasma membranes of the syncytiotrophoblast or paracellularly through putative aqueous channels that penetrate through the syncytiotrophoblast layer (Robinson et al., 1988; Ala-Kokko et al., 1993; Sibley, 1994). As is the case with endothelia and epithelia, the physicochemical properties of drugs play a significant role in determining transtrophoblast permeation. Molecules that are relatively lipophilic, with low protein binding, a low degree of ionization, and have molecular weights of less than 600 d, permeate readily across the syncytiotrophoblast layer (Ala-Kokko et al., 1993). However, the actual molecular weight cutoff for permeation across the placental barrier has been difficult to precisely define. It is known that peptides with molecular weights of ≥1000 d such as oxytocin passively permeates across the placental barrier with sustained maternal blood concentrations (Willis et al., 1986; Malek et al., 1996). Several additional factors influence the overall placental permeation drugs and are summarized in Table 1. These factors include the developmental changes in the placental barrier such as pH and protein gradients, thickness and surface area of the barrier.

3.   Evidence for placental control of permeability and metabolism

The placenta has come to be known as a poor barrier to drug distribution between maternal and fetal circulations (Ala-Kokko et al., 1993; Dancis, 1987). However, in a recent study with a mouse model, Lankas et al. (1998) have provided evidence to suggest that the placenta does limit drug or chemical exposure through expression of a functional multidrug resistant gene product 1 (MDR1a) or P-glycoprotein (Pgp). Similar expression of functional Pgp can also be demonstrated in primary cultures of human cytotrophoblasts which fuse to form syncytiotrophoblasts and the human cell line, BeWo, which is a mixture of predominantly cytotrophoblasts and a few syncytiotrophoblasts (Utoguchi et al., 1998). In the human placenta there is abundant expression of the MDR1 gene throughout pregnancy (Mylona et al., 1996; Allikmets et al., 1998) and specifically in the cytotrophoblast (Mylona et al., 1996; Nakamura et al., 1997). That placental MDR1 is functional in late human pregnancy was questioned by Macfarland et al. (1994) who provided some evidence that the efflux mechanism was seemingly active only early in pregnancy and not always was restricted to trophoblasts. However, subsequent studies with human microvillar membranes of term human trophoblasts have indicated an active MDR1 (Nakamura et al., 1997). The obvious implications for a Pgp at the placental barrier includes the possibility that inhibitors of the efflux mechanism could contribute to chemically-induced teratogenesis; an outcome directly demonstrated when the Pgp was nonexistent in knockout animals.   A key point in understanding the placental barrier from the Lankas et al. (1998) study is that the placenta reduces rather than prevents the drug or chemical exposure of the fetus when one looks at drug concentrations in fetuses of normal animals compared to the fetuses of the knockout animals. Only the knockouts exhibited birth defects. Therefore, one might propose that the use of drugs that are substrates for Pgp would reduce fetal drug exposure (Lankas et al., 1998).

The placenta expresses a number of carrier mechanisms (e.g., amino acid transporters, serotonin transporters, norepinephrine transporters, organic cation transporters, extraneuronal

monoamine transporters, peptide transporters) that are well-characterized in other tissues and are yet, of unknown physiological significance in placental function. Based on accumulating evidence, it is highly likely that a number of drugs of abuse (e.g., cocaine, amphetamines, nictoine, cannabinoids) achieve distribution across the placental barrier by utilizing both passive diffusion pathways and the carrier mechanisms intended for endogenous substrates (Ganapathy et al., 1999).   Consequently, drugs, drugs of abuse, and nutrients may all compete for carriers at the placental barrier and may result in circumstances where fetal exposure to drugs is enhanced at the expense of nutrients.   Thus, our development of a knowledge base on the function of placental transport systems is important for establishing an understanding of the endogenous transport mechanisms that regulate and contribute to fetal drug exposure (Ganapathy et al., 1999).   The characterization of trophoblast biochemical and transport mechanisms has had the benefit of providing a basis for the ongoing investigations directed at targeting gene delivery to the placenta (Parry et al., 1999).

Although largely overlooked, the placenta also expresses cytochrome P450 (CYP) enzymes (Pasanen, 1999) and peptidases (Johnson et al., 1994; Kenagy et al., 1998; Chandorkar et al., 1999). The 1A1/1A2 isoform is the dominant CYP present in the placenta and is inducible to significant activity following exposures to xenobiotics including polycyclic aromatic hydrocarbon components of tobacco smoke.   Additional isoforms, CYP19 (aromatase) and CYP11B (cholesterol side-chain cleaving enzyme) are also known to be present in the placenta throughout pregnancy.   Moreover, the placental has the potential to express an array of CYP isoforms depending on the length of gestation and the health of the mother (Pasanen, 1999). Even less is known of peptidases, although activities of major carboxy- and amino-peptidases also appear to be regulated by development and components of tobacco smoke (Kenagy et al., 1998).

That placental metabolism can influence fetal exposure to drugs and nutrients is not clear.   However, polycyclic aromatic hydrocarbons are substrates for CYP1A1 and exposure to these molecules

correlates with low birth weights in humans and in other species (Khera et al., 1972; Allen and Barsotti, 1976; Shum et al., 1979; Wong et al., 1985). An implication is that placental metabolism regulates nutrient delivery to the fetus. Current speculation is that peptidases play a role in regulating maternal, fetal, and placental derived peptide hormone distribution across the placenta (Kenagy et al., 1998; Chandorkar et al., 1999). The presence of a "metabolic shield" at the placental barrier raises the possibility of considering the design of drugs that are metabolized by the placenta thereby reducing fetal exposure. This is a strategy that has been proposed in developing peptide analgesics for obstetric purposes (Rapaka and Porecca, 1991).

4.   Approaches to investigation of the human placental barrier

A variety of *in vitro* systems currently exists to evaluate the transport and metabolism processes of many of the significant endothelial and epithelial tissue barriers to drug delivery. In fact, many of these systems are presently the primary tools at the drug design and development interface of the pharmaceutical industry. A similar effort or interest in characterizing the placental barrier does not exist.

The emergence of *in vitro* models for the human placenta within the past decade opens the door for investigations of drug transport and metabolism mechanisms and apply that knowledge to developing drugs that more selectively target either the mother or the fetus in drug therapy (Dancis and Liebes, 1995). For example, human placental lobe perfusion systems have shown that mechanisms of placental drug transport rates and extent can be investigated *in vitro* (Bassily et al., 1995; Tuntland et al., 1999; Sastry, 1999). Human trophoblast culture systems, both primary cultures and cell lines, also exist, including those that form monolayers, and have been used to study uptake and transport mechanisms at the cellular and molecular levels (Ringler and Strauss, 1990; Yui et al., 1994; Bloxam et

al., 1997; Liu et al., 1997; Sastry, 1999). A point to be made is that *in vitro* systems are more often derived from mature placenta and may not represent the early pregnancy when drug exposure may be even more critical for the developing fetus. Collectively, the *in vitro* systems have ultimately provided what few details we have on specific mechanisms mediating the distribution of nutrients, hormones, drugs, and drugs of abuse across the human placenta (Malek et al., 1996; Knipp et al., 1999; Ganapathy et al., 1999; Tuntland et al., 1999; Sastry, 1999).

To conclude, the placenta does express some mechanisms that participate in regulating the distribution of therapeutic agents between mother and fetus. Safe and effective use of drugs in pregnancy will depend on the pharmaceutical scientist being able to develop approaches that take advantage of unique placental transport and metabolism mechanisms. Thus, a better knowledge of basic placental physiology and biochemistry through the exploitation of present *in vitro* laboratory techniques, is expected lead to strategies for use of therapeutic agents in pregnancy that do not jeopardize the health of the fetus or the mother.

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Table 1. Primary factors determining drug permeation across the placental barrier.*

Drug properties	*low degree of ionization* *lipophilicity* *low protein binding* *molecular weight* ≈ 600 *d or less*
Placental characteristics	*blood flow* (intervillous maternal and fetal) – influenced by pathophysiological conditions and drugs *concentration gradient* of drug across the barrier *hydrostatic gradient* – slight movement toward the fetal compartment *pH gradient* – slightly acidic fetal compartment *thinning and aging barrier* with advancement of pregnancy *increasing suface area* with advancement of pregnancy *developing metabolism* that is largely unappreciated *protein gradients* – decreasing albumin concentration in maternal compartment with advancement of pregnancy, lower α_1-acid glycoprotein concentration in fetal compartment *nutrient transporters (e.g., amino acid carriers, glucose carriers, monoamine carriers, etc.)* – in addition to passive diffusion, drugs may be transported across the placenta by nutrient carriers
Additional maternal and fetal factors	*fetal growth and development* *fetal metabolism* *fetal tissue binding* *maternal metabolism* *maternal health condition*

*   Information in table summarized from Willis et al., 1986; Dancis, 1987; Ala-Kokko et al., 1993; Stulc and Stulcova, 1993; Moe, 1995; Ramamoorthy et al., 1995; Malek et al., 1996; Ganapathy et al., 1999.