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The human placenta – An alternative for studying foetal exposure

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Abstract

Pregnant women are daily exposed to a wide selection of foreign substances. Sources are as different as lifestyle factors (smoking, daily care products, alcohol consumption, etc.), maternal medication or occupational/environmental exposures. The placenta provides the link between mother and foetus, and though its main task is to act as a barrier and transport nutrients and oxygen to the foetus, many foreign compounds are transported across the placenta to some degree and may therefore influence the unborn child. Foetal exposures to environmental and medicinal products may have impact on the growth of the foetus (e.g. cigarette smoke) and development of the foetal organs (e.g. methylmercury and thalidomide). The scope of this review is to give insight to the placental anatomy, development and function. Furthermore, the compounds physical properties and the transfer mechanism across the placental barrier are evaluated. In order to determine the actual foetal risk from exposure to a chemical many studies regarding the topic are necessary, including means of transportation, toxicological targets and effects. For this purpose several in vivo and in vitro models including the placental perfusion system are models of choice.

Keywords: Human placenta; Development; Functionality; Drug-transport; In vivo methods; In vitro methods; Placenta perfusion

1. Introduction

During the last 50 years evidence has accumulated that many pharmacologic agents and also environmental pollutants from surroundings are transferred from the mother to the embryo or foetus. Regardless of whether this transfer is on purpose as in a medical treatment or unwanted as in women unaware of early pregnancy taking over-the-counter drugs as self-medication, more attention and information for the public is needed (Plonait and Nau, 2004). The first evidence of reproductive toxicity caused by a foetal exposure from maternal intake was the thalidomide disaster in 1957–1961. Pregnant women from approximately 46 countries worldwide were prescribed thalidomide as a safe anti-emetic sedative and anti-anxiety drug. Given between the 34th and 50th day of pregnancy thalidomide can exert teratogenic effects seen as skeletal malformation of especially the limbs in 10,000 surviving babies. When further investigated the mechanism of action of thalidomide was specific in humans but confirmed in animal testing and therefore the demands on testing of drugs to be used in pregnancy were increased to include two or more animal species (Botting, 2002; Brent, 2004). Later, in the early 1970s it was scientifically demonstrated that prenatal alcohol exposure can cause mental retardation, facial malformations, prenatal and/or postnatal growth retardation (West and Blake, 2005; Riley and McGee, 2005). In 1971 the reproductive damaging effect of the synthetic nonsteroidal estrogen diethylstilbestrol (DES) became evident. The drug was prescribed to prevent miscarriage and other pregnancy complications but as an unknown teratogenic effect, it also caused carcinomas in vagina and cervix in young women offspring and malformation of reproductive organs in both girl and boy offspring (Swan, 2000). This led to a broader definition of reproductive damaging effects, including not only functional and cognitive effects seen at birth, but also effects seen later in life caused by a foetal exposure. It is now common knowledge that maternal smoking

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(Habek et al., 2002), and exposure to methylmercury, lead, environmental chemicals as polychlorinated biphenyls (Schantz, 1996; Tilson et al., 1998) may cause negative health effects in the human offspring. The scientific communities and public now pay more attention to the potential teratogenic and foetotoxic effects with increased focus on foetal exposure.

In this review, we will give a short introduction to the placenta and its function. Furthermore, the mechanisms allowing drugs and substances to cross the placental barrier will be described, and different methods to investigate the placental transport and toxicity of endogenous compounds. Emphasis is on the placental perfusion system as it is an interesting tool for current studies of drug transfer.

1.1. Placenta anatomy and development

The human placenta is unique in structure (anatomy, pathology, and physiology) and only resembles placenta from certain primate species, e.g. the macaque (Enders and Blankenship, 1999).

The placenta is defined as the fusion of foetal membranes with the uterine mucosa for the purpose of maternofetal exchange of nutrients, gases, and waste substrates. According to this definition, placental development starts at day 6 or 7 post-conception, as soon as the blastocyst starts invasion of the endometrium. The blastocyst consists of an outer cover, the trophoblast, and an inner cell mass, the embryoblast (Kaufmann and Frank, 2004). The trophoblast cell lineage originates from the trophectoderm at the blastocyt stage and the stromal and vascular components of the placenta are derived from the allantois, also of fetal/blastocyst origin (Cross, 2006). It is parts of the trophoblast of the blastocyst that adheres to and invades the uterine mucosa and in turn differentiates to the syncytiotrophoblast. The primary drug transport site in the grown placenta, the villi, is developed during the lacunar period (day 8–13 post-conception). The villous system consisting of trabeculae (separating outgrowths becoming pre-villi) and lacunae (vacuoles) arise in the syncytiotrophoblast. Surrounding this system is the primary chorionic plate (towards the embryoblast) and the trophoblastic shell (towards the endometrium). The pre-villi are formed by the cytotrophoblast that invades the trabeculae from the primary chorionic plate and proliferates inside of these resulting in branches that protrude into the vacuoles. If the villi are attached to the trophoblastic shell (basic plate) they are called anchoring villi. The still expanding lacunae system becomes the intervillous space. The first primitive maternal circulation is established by cells from the trophoblastic shell which manoeuvre themselves into the maternal endometrial vessels (from day 12 post-conception). The maternal blood enters the lacunar system through small holes in the shell (Syme et al., 2004; Kaufmann and Frank, 2004). From the chorionic plate mesenchymal cells invade the primary villi thereby making it secondary villi. Some of these invasive mesenchymal cells are differentiated into hemangioblastic cell cords which further differentiate into the first foetal capillaries (tertiary villi). A population of the same hemangioblastic cell cords differentiates into hematopoietic stem cells which start the blood formation inside the capillaries. At the same time the fetally vascularized allantois reaches the chorionic plate and extends through the plate into the villi where they fuse with the intravillous capillary bed. The result is the intraplacental foetal circulation which is fully established at the end of the fifth week post-conception (Kaufmann and Frank, 2004). However, the complete foetal–placental–maternal circulation is not entirely established until around the tenth week of pregnancy, therefore substances present in the maternal blood until this time must be introduced to the embryo via diffusion through the extracellular fluid (Syme et al., 2004). The foetal circulation ends in the villous trees and these are found in the vascular units (cotyledons) within the placenta. The full-term placenta contain between 10 and 40 cotyledons separated from each other by the placental septa.

The Grosser classification is still widely used as a mean to characterize placenta. Placentae described by the absence of maternal tissue such that maternal blood directly contacts the trophoblast are called haemochorial (humans, monkeys). A schematic representation of the full-term haemochorial placenta is given in Fig. 1. The placental barrier in this type is composed of tissue from foetal origin only. Placentae called endotheliochorial (cat, dog) have brought the trophoblast into contact with the maternal capillary endothelium due to erosion. Epitheliochorial (horse, pig) has six layers of tissue separating the foetal and maternal circulations. Finally, in syndesmochorial (ruminants) placentae the maternal endothelium disappears, resulting in trophoblastic cells in direct contact with

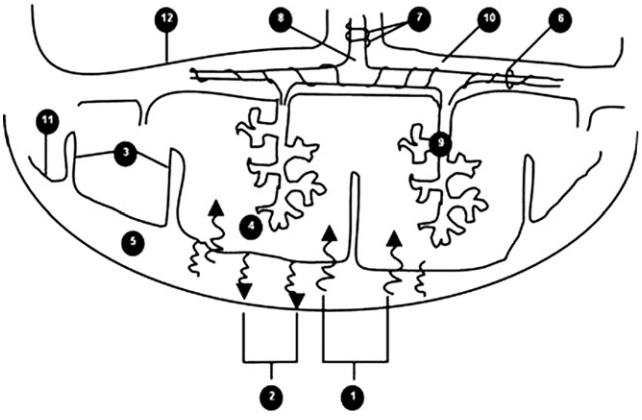

Fig. 1. Schematic drawing of a transverse section through a full-term placenta. Oxygen-rich blood is pumped from the maternal circulation into the intervillous space. Exchange of nutrients, waste products, gases and xenobiotics takes place across the villus tree, thereby the blood becomes oxygenated and is transported to the foetus via the umbilical vein. (1) Endometrial arteries (maternal circulation), (2) endometrial veins (maternal circulation), (3) placental septa, (4) intervillous space, (5) decidua basalis, (6) chorionic plate, (7) umbilical arteries (foetal circulation), (8) umbilical veins (foetal circulation), (9) villus tree, (10) syncytiotrophoblast, (11) cytotrophoblast, and (12) amnion.

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maternal connective tissue (Grosser, 1909; Faber et al., 1992; Leiser and Kaufmann, 1994; Simone et al., 1994). During human pregnancy, the foetal and maternal circulations are separated by the placental barrier that consists of five layers in the first trimester: the syncytiotrophoblast layer (lining the villi), the cytotrophoblast, the trophoblastic basal lamina, connective tissue, and the foetal endothelium. This barrier undergoes drastic changes throughout pregnancy: the syncytiotrophoblast layer is largely reduced in thickness, the cytotrophoblast becomes discontinuous, changes in the villus structure are also found; it enlarges probably to ease the exchange-processes between mother and foetus (Fox, 1991; van der Aa et al., 1998; Kaufmann and Frank, 2004). Both the differences in trophoblastic layers and the anatomic differences of the villous trees between species result in a large variation in placental function. Factors as diffusion, electrical potential across the barrier, magnitude of maternal and foetal blood flows, and the possible differences in presence of carriers should be considered. Consequently, placental function between species is different, especially the transfer and metabolism of drugs vary considerably (Simone et al., 1994; Syme et al., 2004).

1.2. Placental function

The placental function varies during pregnancy, thus during early gestation the primary function of the early placenta is to mediate implantation of the embryo into the uterus and, the secondary function is to produce hormones that prevent the end of the ovarian cycle. After implantation, the primary placental function is to regulate nutrient and oxygen uptake from the mother to the foetus. The placenta plays an active role in regulating maternal physiology to the nutritional benefit of the foetus, e.g. the trophoblast produce angiogenic factors and vasodilators, produce hormones that stimulate the maternal blood cell production and blood volume, produce growth hormones and placental lactogens and hormones that suppress and stimulate appetite, etc. (Cross, 2006). These substances and other drugs are transported across the placenta via specific transport mechanisms present in the maternal-facing apical (brush border) membrane and fetal-facing basal membrane of the syncytiotrophoblast (Syme et al., 2004).

It is well established that the placenta metabolizes and transfers a large diversity of pharmacologically active molecules. Therefore, some concern rests in if the placental metabolism converts precursors into potential toxic metabolites. Though, the metabolizing system has metabolic activities and a substrate spectrum seemingly somewhat reduced when compared to the liver, the placenta contains a rich enzymatic machinery able to carry out both Phase I and II reactions (Pasanen and Pelkonen, 1994; Pasanen, 1999; Marin et al., 2004). Several cytochrome P450 proteins including CYP1, CYP2 and CYP3 have been isolated from the placenta. These proteins are largely responsible for the mechanisms in the detoxification of drugs and toxins. The enzymatic machinery has been thoroughly reviewed in Pasanen and Pelkonen (1994) and Marin et al. (2004).

1.3. Placental transport mechanisms

The speed and the extent of compound-transfer depend on the physiochemical and structural characteristics of the drug as well as the physical characteristics of the maternal–placental–embryonic-foetal unit. The physiochemical characteristics of a compound can determine their transfer rate through the placenta, considering the weight, ionization and lipid-solubility of the compound. Molecules with a weight up to 600 Da, non-ionized and lipid soluble will show unimpeded diffusion. The transfer rate is called flow-limited transfer and will depend only on the factors regulating maternal and foetal blood flows. Larger, ionized, hydrophilic compounds will cross the placenta more slowly. Transfer of this type of compounds is referred to as membrane limited transfer and the rate is slower than the blood stream. The constituents of the membranes determined the transfer rate (van der Aa et al., 1998). Hydrophilic molecules encounter a resistance by the haemocordial type of placenta due to the trophoblast and the endothelium (Thornburg and Faber, 1977; van der Aa et al., 1998). The physical factors include the surface area of the exchange membrane; the thickness of the endothelio-syncytial membrane; the maternal blood flow and the hydrostatic pressure in the intervillous chamber; the blood pressure in foetal capillaries and the difference in the osmotic pressure between mother and foetal compartments (Bourget et al., 1995). Closer to term-pregnancy the exchange between mother and child intensifies partly because of the thinning of membranes as described above.

The transfer of compounds can occur by four kinds of mechanisms. First, many compounds will diffuse across the human placenta by the passive diffusion process. This process is a transfer without the use of energy. It is dependent only by the factors introduced in Fick's law of passive diffusion: rate of diffusion $= D \times \Delta c \times A/d$; the surface area ($A$); thickness of the membrane barrier (d); the drug concentration gradient across the membrane (between the maternal and fetal blood) (Δc) and the substance specific diffusion constant (D) (Plonait and Nau, 2004). Importantly, the compound characteristics and protein binding capacities also influence the substance's capability of crossing the placenta. Many pharmacologically active compounds cross the placenta by simple diffusion, although also larger molecules as antibodies may be transported this way (Malek et al., 1998). Second, the transport of the substance is carrier mediated down a concentration gradient without energy-costs called facilitated diffusion. Only a few drugs have been suggested to be transported this way, one of them is ganciclovir against intrauterine infection. Ganciclovir was shown to be taken up by the maternal-facing placental membrane by a carrier-dependent, Na-independent system (Henderson et al., 1993). Generally,

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compounds structurally related to endogenous compounds intended for this kind of transport, are assumed to use facilitated diffusion, e.g. hormones and nucleosides (van der Aa et al., 1998; Syme et al., 2004). Third, active transport includes the movement of a substance against a chemical or electrical gradient with energy costs. The transport is carrier mediated and there is a high degree of competition between related compounds. An example is the sodium/multivitamin transporter (SMVT) located in the placental brush-border membrane. It has been discussed whether the drugs like carbamazepine compete with endogenous biotin for the SMVT transporter (Ganapathy et al., 2000). Fourth, pinocytosis in which the compound is invaginated into the cell membrane where it is transferred to the opposite site as a vesicle. However, the current conclusion of many placental transport studies is that this process is too slow to be highly relevant for the transfer of drugs from mother to foetus (Syme et al., 2004).

Up to now, approximately 20 different drug transport proteins have been determined (Unadkat et al., 2004; Myllynen et al., 2005). However, it is not clear whether these transporters are coupled to transport of foreign chemical substances – thus they do have the potential. One example is the antiepileptic drug gabapentin that inhibited LAT1-mediated [^{14}C]phenylalanine uptake in a competitive manner (Uchino et al., 2002; Myllynen et al., 2005).

2. Models to study placental transfer

So far, very few drugs have been proved teratogenic in humans when used in clinically effective doses (Webster and Freeman, 2001). Therefore, the fast expanding list of everyday life chemicals from daily care products, food, pollution particles, toys, etc. may prove to have unwanted effects on the developing foetus. In addition, epidemiological studies of such environmental compounds are even more difficult due to complex mixtures and low concentrations.

Due to obvious ethical reasons, risk assessment studies of foetal development from maternal exposures to chemicals and pharmaceuticals are not performed in humans. Most commonly risk assessment is based on results from animal studies sometimes supported by in vitro studies. As implied above it is imperative that these studies are performed to test the toxicology and mechanisms of action of drugs and chemicals, and to determine the safe doses in humans. When it comes to the unborn child many voices argue that the foetus is most vulnerable to toxicants while others claim that the opposite might be the case, or at least that it depends on the chemical and the animal tested (Brent, 2004). Thus, it is of utmost importance to study the transfer of chemicals and drugs across the placenta, and researchers need to investigate approaches to obtain the data concerning agents that have not yet been comprehensively studied. In this section a summation of the current methods to investigate the placental transfer is available.

2.1. In vivo models

Many in vivo models concern the use of pregnant animals to determine the risk of birth defects and other reproductive effects. Animal teratology studies are no doubt helpful in determining the reproductive effect of a drug or chemical, but animal models with regard to relevance to human kinetics may be limited, because of difficulties in extrapolating the experimental results to humans (Nau, 1986; Botting, 2002). When testing only the transfer of a compound, primates such as rhesus macaques and baboons have been used since they have haemochorial placentation similar to the human. In a study by Patterson et al., it was shown that the anti-HIV compound, d4T, and its active and inactive metabolites were transferred across the rhesus macaques late term placenta (Patterson et al., 2000). In autoradiography studies, it is possible to investigate the distribution of radiolabeled compounds. In older studies it has been possible to perform this kind of study in pregnant mice and rats. Thus revealing if the compound was transferred to the foetus and target organs with the largest both foetal and maternal concentration might be found (Brittebo et al., 1994).

Interestingly, the current OECD test guidelines for reproductive toxicity testing include prenatal developmental studies (TG 414), one-generation studies (TG 415), two-generation studies (TG 416), and a reproduction/developmental toxicity screening test (TG 421 + TG 422). These studies are designed only to provide information concerning the toxic effect of prenatal exposure on the pregnant test animal, on the developing organism in uterus, and the integrity- and performance-effects of the male and female reproductive systems after one or two exposed generations. Fertility, growth, malformations, and survival are typical effect endpoints after administration of very high doses. Analysis of the distribution of compounds in and between maternal and foetal compartments is not required in all instances. However, this kind of information would certainly contribute to the understanding of the toxicokinetics of a compound.

2.1.1. Foeto-maternal (F/M) blood concentration ratio

Sampling of human maternal and cord blood at the same time and further analysis for presence of compounds is a fairly simple and ethically agreeable method. This technique is used to illustrate the drug concentration between the two circulations. The method can be further expanded; first to sampling from both the artery and vein from the cord, and thereby additional information as the steady-state rate and directional transfer can be determined (Bourget et al., 1995), second it is possible to perform placenta biopsies hereby providing information on the materno-placental or foetal–placental concentration ratios. Sampling of blood from both mother and foetus can be performed only once at a particular gestational age which can be extremely important should medical complications occur during the pregnancy, e.g. chorioamniotitis (Bourget et al., 1995; Sas-

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try, 1999). However, even though the method provides an exact answer to an immediate problem no information as to the distribution of the drug in tissue is available. Thus, concerns of e.g. metabolic fate and accumulation in foetal compartments remain unanswered.

2.1.2. Coelocentesis

An alternative to foetal blood sampling was introduced in 1991 (Jauniaux et al., 1991). The study of human coelocentesis is another method by which it is possible to obtain information of the transfer across the early placenta. With this technique samples from the human exocoelomic and amniotic fluid can be retrieved by transvagianal puncture from week 6–10 and week 7–12, respectively. Protein metabolism of the early placenta has been studied in this system, e.g. transport of cotinine across the early placenta has been shown (Jauniaux et al., 1991; Jauniaux and Gulbis, 2000). The coelocentesis method, however, do not allow use of kinetic models based on blood and plasma samples. Kinetic models are necessary to allow for extrapolating quantitative drug transfer to the foetus and subsequent risk assessment (Plonait and Nau, 2004).

2.2. In vitro models

In vitro models have the potential of replacing or reducing the number of animals used for toxicological testing. Their results may provide important knowledge about the transplacental transfer in humans of new chemical substances as well as environmental exposures of hazardous compounds. Though in vitro models cannot fully account for all the physiological and biochemical variables in the mother, placenta and foetus and how these variables change throughout gestation, they should be the first in line when a new substance is to be investigated (Syme et al., 2004).

2.2.1. Monolayer cell cultures

A model of the villous syncytium from primary cytotrophoblasts forming tight-junctions designed to study placental barrier function has been reported (Hemmings et al., 2001). This type of model is excellent for prolonged studies of the placental barrier. However, the method is difficult since the isolation of cytotrophoblast cells has a high contamination level and the cell cultures are not viable for more than one week (Hemmings et al., 2001). To our knowledge, this method has not yet been used to test drug transfer, but investigations involving parasites, virus and apoptosis signalling have been performed by the Guilbert group (Hemmings et al., 1998; Abbasi et al., 2003; Kilani et al., 2007).

Primary cell lines of a wide variety can be produced from the human placenta. By using gentle digestion preparations mostly syncytial trophoblasts can be and have been obtained and cultured (Sullivan, 2002). Since the primary cell lines are not viable for many passages, the immortalized cell lines described below are often used in their place.

2.2.2. Cell lines from human placenta

Generally speaking there are three main types of cell lines derived from the placenta; those which spontaneously arise from cultured cytotrophoblast in vitro, those immortalized by viral transfected genes, and those from spontaneous choriocarcinomas (for review of these groups turn to Sullivan, 2002). Of these three types, cells from the third category are most often used; as cells from human choriocarcinoma display many of the biochemical and morphological characteristics reported for in utero invasive trophoblast cells (Wadsack et al., 2003). Human placental choriocarcinoma cells, BeWo, JAr and JEG cells share many of the same properties but noteworthy differences in characteristics have been identified (King et al., 2000; Sullivan, 2002; Vahakangas and Myllynen, 2006). Grown in inserts (e.g. fibronectin coated) they are relatively easy to use as a model mimicking the placental barrier by letting the cells form a monolayer or multiple layers of cells (Liu et al., 1997). Consequently, they are commonly used in toxicology to study transplacental transport and metabolism in vitro. The downside to these cell lines is an ongoing discussion of whether these cells express the same markers as their origin or slightly different markers (King et al., 2000).

2.2.3. Placental explants

Tissue explant studies started more than 50 years ago and have gradually been improved since then (recently reviewed by Miller et al., 2005). They have a wide variety of use, such as placental transport, metabolism, endocrine function, enzyme function, cellular proliferation and differentiation. Tissue explants has been employed from both early gestation and near-term placenta. Placental explants offer the advantage of intact microarchitecture and maintenance of cell–cell interactions and paracrine communications; hence the contribution of mesenchymal and endothelial cells to any metabolic processes can be taken into account (Syme et al., 2004). They have a lifetime of up to 11 days, but users should be careful and monitor any damages to the explant (Miller et al., 2005).

2.2.4. Placental microsomes

Placental microsomes are used as an enzyme activity model thereby providing means for studying chemical metabolism in the placenta. Microsomes can be prepared from placenta by differential centrifugation (Ostrea et al., 1989), thus this system can be used to address both early and late placental enzyme activity. It has been reported that microsomal studies demonstrate large inter-individual differences in metabolizing activities (Vahakangas and Myllynen, 2006).

2.2.5. Membrane vesicles

Isolated membrane vesicles from the placental trophoblast provides an experimental system where transport mechanisms from both the foetal and the maternal plasma membrane can be examined separately (Murer and Kinne, 1980; Bissonnette, 1982). Though useful for studying basic

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transport mechanisms, the vesicles only provide information on a piece of the puzzle. The disadvantage of the system is that membrane transporter activities usually are measured in the absence of regulatory factors and therefore do not reflect the in vivo situation.

2.2.6. Ex vivo perfused placenta cotyledon

Human placental perfusion test system offers information about transplacental transfer, placental metabolism, storage, acute toxicity and potential role of transporters, vascularization, and foetal exposure. The perfusion of the isolated human placental cotyledon was first described in 1967 by Panigel et al., later modified by Schneider, Miller and other research groups to enable more systematic studies (Panigel et al., 1967; Schneider et al., 1972; Miller et al., 1985). Perfusion of placental tissue provides an accurate ex vivo model of substance or drug movement from the maternal to the foetal side of the syncytiotrophoblast (Hemmings et al., 2001) or to determine clearance from the foetal circulation after drug-treatment of the mother (Sastry, 1999). To date this system has been validated by a number of transfer studies using different substances. Summarizing tables concerning penicillins, cephalosporins, aminoglycosides and antibiotics was recently reviewed by Pacifici (2006). Bourget et al. presented a list of drugs tested in the perfused cotyledon model from 1972 to 1994 with therapeutically interest (Bourget et al., 1995). Table 1 presents the drug and disease inhibitors tested in the placenta perfusion system from 1995 to 2006 to give an idea of how far this research area has come. The perfusion model has been used to validate as different substances as HIV protease inhibitors and anti-epileptic drugs. In the case of the drug saquinavir, it was indicated that use of the retroviral drug by pregnant women may not lead to significant exposure of the foetus (Forestier et al., 2001). In contrast, the drug lamotrigine crossed the placenta easily and rapidly leading to a considerable foetal exposure (Myllynen et al., 2003).

The use of placenta in scientific research causes a minimum of ethical problems partly because the experiments are non-invasive, causing no harm to mother or child, and partly because placentas are discarded and incinerated after birth. The practical procedures of the perfused cotyledon model start in obtaining a human term placenta after an uncomplicated birth, which involves a signed declaration of informed consent from the mother. The placenta is then perfused with heparinized Krebs–Ringer bicarbonate buffer. A foetal artery-vein pair supplying a well-defined cotyledon without leaks is cannulated. The cotyle-

Table 1
Placental transfer studies of disease inhibitors and drugs in the human placental perfusion system from 1995 to 2006

Category	Substance	Transfer (+/−)	Reference
Abused agents	Cocaine	+	Malek et al. (1995)
	Morphine	+	Kopecky et al. (1999)
	Nicotine	+	Pastrakuljic et al. (1998)
	Nicotine and cotinine	+/+	Sastry et al. (1998)
	Cocaethylene	+	Simone et al. (1997)
	Methadone	+	Nekhayeva et al. (2005)
HIV inhibitors	Saquinavir	−	Forestier et al. (2001)
	AZT	+	Olivero et al. (1999)
		+	Boal et al. (1997)
	Lopinavir/ritonavir	+	Gavard et al. (2006)
Therapeutic agents, antidepressants, antibiotics	Amitriptyline	+	Heikkinen et al. (2001)
	Trovafloxacin	+	Casey and Bawdon (2000)
	Ciprofloxacin, ofloxacin, levofloxacin	+/+/+	Polachek et al. (2005)
	Vancomycin	−	Hnat et al. (2004)
Antidiabetic-related drugs	Rosiglitazone	−	Holmes et al. (2006)
	Desmopressin	−	Ray et al. (2004)
	Glyburide	−	Nanovskaya et al. (2006)
	Insulin lispro	−	Boskovic et al. (2003)
		−	Holcberg et al. (2004)
Anaesthetics	Lidocaine	+	la-Kokko et al. (1995)
	Methohexital	+	Herman et al. (2000)
	Ropivacaine and bupivacaine	+/+	Johnson et al. (1999)
	Fentanyl, alfentanil, sufentanil	+/+/+	Giroux et al. (1997)
Epilepsy drugs	Oxcarbazepine and carbamazepine	+/−	Pienimaki et al. (1997)
	Lamotrigine	+	Myllynen et al. (2003)
Pregnancy related treatment	Hydralazine	+	Magee and Bawdon (2000)
	Methimazole and propylthiouracil	+/+	Mortimer et al. (1997)
Heart condition	Digoxin	+	Schmolling et al. (1997)

+ indicates a positive transfer and − indicates low or no transfer compared to the reference (e.g. antipyrine).

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don is separated from the placenta and placed in a plexiglas perfusion chamber where placing cannulae through the decidual plate simulates the maternal circulation. The cotyledon is perfused via two peristaltic pumps, each connected to a reservoir containing a buffer. After a 30-min pre-perfusion period the chemical and the reference substance antipyrine can be added to either the maternal or foetal compartment. A schematic diagram of the model is shown in Fig. 2. The maternal perfusion solution is in permanent contact with a 95% O_2/5% N_2 gas mixture. For short experiments (2–4 h) a Krebs–Ringer buffer added glucose and salts can be used, but for longer perfusions (6–8 h) a cell culture medium is needed. Experiments can be performed by using either closed (re-circulating) or the open (non-circulating) method. Re-circulating studies imitate physiological conditions and can be used to study transplacental transfer and metabolism of the compound. In open perfusions drug clearance can be studied (Bourget et al., 1995; Sastry, 1999; Vahakangas and Myllynen, 2006; Mose and Knudsen, 2006). The placenta perfusion model is often praised as a non-invasive and ethical method. Though, it is probably one of the best known methods for validating transfer of substances known to be toxic to the foetus, it has shortcomings (see below). The cells in perfused placentae remain viable for up to 48 h, therefore this model is suitable for many types of investigations, but fails when it comes to longer perfusion times, e.g. transmission of infectious diseases (Hemmings et al., 2001). Thus, the perfusion ex vivo can be followed for several hours, allowing the monitoring of the initial kinetics of a substance passing the maternal–foetal threshold. Whether this is enough time to reach an equilibrium enabling conclusions about the maternal/foetal ratio of a compound concentration over a prolonged period of time is questionable. Unpublished results from our group indicate that the question might be partly determined by the specific properties of the chemical under investigation.

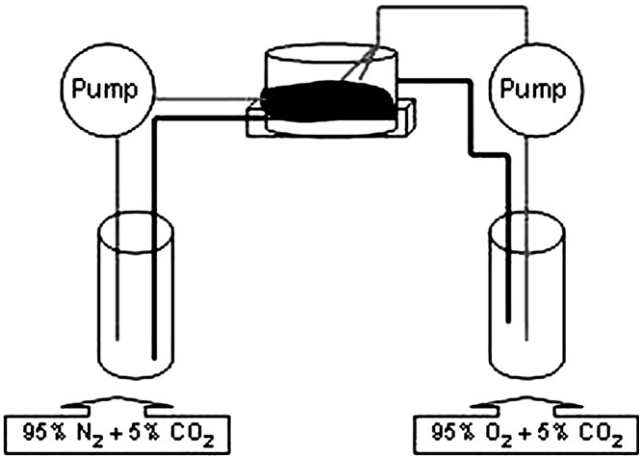

Fig. 2. Schematic presentation of the placenta perfusion system. The maternal and foetal circulations are driven by two pumps. The foetal perfusate is gassed with 95% N_2/5% CO_2 and the maternal perfusate is gassed with 95% O_2/5% CO_2.

Extrapolation of data obtained from the placenta perfusion model is not straightforward. The system is metabolically stable, which is unlike the pregnancy state (Bourget et al., 1995). A full-term placenta is used, which means that some of the transporters and the metabolism are different in the first trimester placenta vs. the full-term placenta (Vahakangas and Myllynen, 2006). It might also be that at term the placental transport mechanisms and metabolism within the tissue may have ran down, and thereby the method underestimates the contribution during pregnancy. Furthermore, the trauma the placenta undergoes during delivery may have effects on the results (Sastry, 1999). The model has been questioned as not being a good representative of the pre-term foetus, since the early foetus might be more susceptible towards toxic hazards (Bourget et al., 1995). However, since the placental thickness and the number of cell layers decrease towards the end of pregnancy, it is possible that the term placenta might be more sensitive to environmental agents than first anticipated (Vahakangas and Myllynen, 2006). In any circumstance the model does not represent the first trimester placenta. Another point of criticism is that this type of model does not allow the observation of parameters, such as blood flow (important for rapidly transferred compounds) or plasma–protein binding (Bourget et al., 1995).

When performing drug toxicology studies on placental passage of a drug or chemical, a part of it may be metabolized by placental metabolic enzymes. This information should be retrieved from data relating to the transplacental passage of the chemical (Sastry, 1999).

3. Conclusions

The pregnant woman is exposed to a still expanding list of chemicals and thereby also her growing foetus. As this list grows the use for proper evaluation of the efficacy of the drugs during pregnancy and their foetal effects needs to be investigated to evaluate risk assessment. The focus of this review has been to explain the placental anatomy and how many complicated processes that underlie transfer of a compound from mother to foetus. Both in vivo and in vitro investigations can help in providing knowledge of the transfer and metabolism of a drug across the placenta. When choosing a method to study transfer or metabolism it is a great advance to use human tissue when possible.

Effect-studies in animals indicate that many chemicals have the potential to cause reproductive toxic effects in human, e.g. thalidomide. When extrapolating toxicological results from laboratory animal to human the differences between species must be taken into account. Differences include parameters like kinetics, placenta, sensitivity, time of birth, and background level. Therefore, it is beneficial to use human placenta tissue as extrapolations from animals to human are bypassed. The human placenta perfusion system is one of many in vitro methods that will work well as a supplement to the existing animal test used for human foetal health risk assessment. Results from

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human in vitro studies can improve the human foetal exposure assessment providing the important information on placental toxicokinetics. Such data are seldom included in reproductive toxicity studies in animals. The best understanding of how substances are transported across the placental barrier must come from combined studies with both in vitro and in vivo investigations.

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