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MiniReview

Modelling of Human Transplacental Transport as Performed in Copenhagen, Denmark

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Abstract: Placenta perfusion models are very effective when studying the placental mechanisms in order to extrapolate to real-life situations. The models are most often used to investigate the transport of substances between mother and foetus, including the potential metabolism of these. We have studied the relationships between maternal and foetal exposures to various compounds including pollutants such as polychlorinated biphenyls, polybrominated flame retardants, nanoparticles as well as recombinant human antibodies. The compounds have been studied in the human placenta perfusion model and to some extent *in vitro* with an established human monolayer trophoblast cell culture model. Results from our studies distinguish placental transport of substances by physicochemical properties, adsorption to placental tissue, binding to transport and receptor proteins and metabolism. We have collected data from different classes of chemicals and nanoparticles for comparisons across chemical structures as well as different test systems. Our test systems are based on human material to bypass the extrapolation from animal data. By combining data from our two test systems, we are able to rank and compare the transport of different classes of substances according to their transport ability. Ultimately, human data including measurements in cord blood contribute to the study of placental transport.

With our work, we contribute to the paradigm shift in toxicology research from the use of animal studies towards alternative models with high relevance to human beings. Modes of actions, exposures and analysis can be studied with modelling, new omics technologies and *in silico* testing, preferably including study material of human origin [1,2]. Replacement of animal use in this area calls for human mechanistic and human toxicity pathway analysis bridged with exposure information as currently developed in the USA in the report *Toxicity Testing in the 21st Century: A Vision and a Strategy* [3], which also includes transport studies across cellular barriers such as the placenta.

Risks from prenatal exposure to environmental toxicants are highly dependent on placental transport of substances from the maternal circulation to the foetal circulation. The presence of a substance in human cord blood is the ultimate demonstration of transport during pregnancy. Placental transport kinetics can be investigated by using the human dually perfused recirculating placental perfusion model and the BeWo cell transfer model.

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In the placental perfusion model, a human term placenta is obtained directly after birth and a single cotyledon is reperfused in the laboratory set-up. Foetal and maternal circulations are re-established by a pump system, and the transport of a chosen test substance can be investigated. This perfusion model is a simplified model of placental transport, and it does not take all the physiological and biochemical variables in the mother and foetus into account. The model can only represent transport in the late third trimester. However, the assets of the model are that the *in vivo* placental metabolism is still intact and that assessment of substance binding to placental tissue can be investigated [4,5]. Placental perfusion models are primarily used to investigate the exchange of substances between mother and foetus, including the potential metabolism of these. It is also used to study the effect of different factors on the placental tissue, including viability, hormone production alterations and the accumulation of substances in the placenta.

The BeWo clone b30 human choriocarcinoma cell line can be cultured as confluent monolayers convenient for directional transport studies [6]. These trophoblast cells represent the rate-limiting barrier for maternal–foetal transfer, and the cell line can also be used to study mechanisms of placental transport and metabolism [7–9].

Both models are under constant development to represent the *in vivo* situation as closely as possible.

Human placental transfer can also be investigated *in vivo* by comparing the concentration of a substance in a maternal blood sample taken near the time of delivery to the concentration of the substance in the umbilical cord blood. This is only possible when investigating endogenous or ubiquitous environmental substances, medication or other exposures. These blood samples present a true picture of transfer and exposure, but do not display the placental transfer kinetics.

The Recirculating Dually Perfused *Ex Vivo* Human Placenta Model

The recirculating dually perfused *ex vivo* human placenta model for estimating foetal exposure is used in laboratories worldwide, and we adapted the model in Copenhagen from the models described by Schneider [10] and by Myllynen *et al.* [4]. The model has been functioning at our facilities since 2004, when the collaboration with the maternity ward at the Copenhagen University Hospital (Rigshospitalet) was established, and the logistics and the system prototype were set up. Since then, improvements in the equipment, handling of placenta and the use of different perfusion media have been implemented as a result of international collaboration and PhD programmes [11]. Maternal consent is always given before or in relation to the birth, and only term placentas from uncomplicated pregnancies is used. On the day of the perfusion study, the placenta is received from the midwife directly after birth, where 40–60 mL of room temperature Krebs Ringer buffer supplemented with glucose and heparin is injected into the two umbilical arteries. The placenta is immediately transferred to the perfusion laboratory where a suitable artery–vein pair that supplies one cotyledon is cannulated with neonatal feeding tubes and cut out in a circular section of 9 cm in diameter to fit into the perfusion chamber (fig. 1).

The perfusion chamber is transferred to a 37°C heated flow bench, where tubes supply the foetal vessels in the single cotyledon with fresh 37°C Krebs Ringer buffer supplemented with glucose and heparin at a rate of 3 mL/min. The maternal circulation is established by blunt cannulation of the maternal side of the placenta, flushing the tissue in the cotyledon surrounding the foetal vessels with maternal perfusion medium. At this point, the foetal supply of fresh Krebs Ringer buffer is replaced by a recirculating reservoir of perfusion medium. The foetal and maternal recirculating perfusion media (each 100 mL) consist of cell culture medium (RPMI 1640, Panum Institute, University of Copenhagen) supplemented with 1 mL L-glutamine (200 mM), penicillin and streptomycin (1%), and heparin (5000 IU/mL, Copenhagen University Hospital Pharmacy, 5 mL/L medium). Ideally, the placental perfusion model should mimic normal physiological conditions, for example, in regard to plasma proteins and cellular constituents of foetal and maternal medium. However, the addition of full blood with erythrocytes would necessitate the use of large quantities of anticoagulants like heparin, and it would not be feasible to obtain the large amount of blood needed for perfusion. The effect of albumin on the transport of the chosen test substance can be investigated by comparing the results from perfusions with or without medium containing human serum albumin (HSA). Since foetal albumin levels at term are higher than maternal levels *in vivo* [12], and to represent the higher viscosity of foetal blood, 40 mg/mL albumin is used in the foetal perfusion medium and 30 mg/mL in the maternal medium.

The transfer of oxygen from the maternal to the foetal compartment is used as a marker of contact between the maternal and the foetal vessels in the perfused areas. This marker is most useful at the establishment of each perfusion to resituate the maternal cannulae in case of insufficient contact, as applied by Wier and Miller [13]. Oxygen transfer together with glucose consumption and lactate production is metabolic measurements ensuring tissue viability rather than tissue integrity during perfusion [13]. It has been demonstrated that the placenta has a large tolerance to hypoxia and recovers well from the ischaemic period from delivery to re-perfusion in the laboratory [14]. The placenta presumably enters a state of partial metabolic arrest after delivery with energy-saving mechanisms and down-regulated metabolism, similar to what is observed in hibernating mammals or deep-sea diving turtles [14–16]. Further studies are, however, needed to clarify the consequences of partial metabolic arrest upon re-perfusion and extrapolation of the results to the *in vivo* situation. Studies of the effect of hypoxia on the metabolism of placental explants [17,18] have shown changes in the metabolic footprint of the explants with regard to oxygen concentration in the incubation media and differences between the metabolomics of placentas from normal and pre-eclamptic pregnancies.

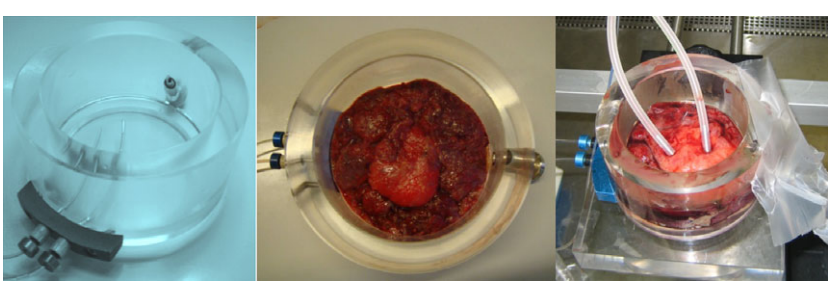

Fig. 1. Perfusion chamber with a solid surface and gravitational exit of maternal perfusion medium in the wall of the perfusion chamber; the foetal vein and artery tubes are inserted through the wall of the chamber.

The positive control antipyrine is added to all perfusions, to ensure sufficient overlap between maternal and foetal systems. Several parameters are needed when presenting placental perfusion data. The foetal/maternal (FM) ratio perfusion curve is most often presented for the study compound and antipyrine. The FM ratio is sometimes supported by a calculation of an area under the FM ratio curve (AUC) between the beginning of perfusion and a specified perfusion time. The transfer index (TI) is sometimes used as a way to normalize the data combining the percentage transfer of study compound in relation to the percentage transfer of antipyrine at, for example, 120 min of perfusion. However, FM and TI may result in misleading conclusions when the study compound accumulates in the tissue or in the perfusion system. Therefore, the data presentation should be supported by a calculation of the recovery of the study compound after the end perfusion, or the concentration in foetal and maternal circulations should be presented separately.

The variation in transport (S.D.) between the perfusion experiments is an indication of individual variation between cotyledons and individuals. This variation in transport between placentas demonstrates the ability of the placenta perfusion model to take into account the individual differences in the *in vivo* human placental transport of substances, representing the true physiological range. On the other hand, this individual variation can cause small differences in substance transport to be more difficult to show, especially if the substances are not studied in the same placental perfusion.

Use of human samples implies protection of sensitive data to encourage donation without the risk of stigmatization of the donor. Any donation must be voluntary and upon the receipt of written and oral information confirmed by written consent. All information about the donor and any results from the studies of the placenta and blood must be kept confidential and only made accessible to third party in a coded, non-identifiable format and upon ethics approval by competent local authorities. Placental tissue is normally discarded after inspection of the placenta for abnormalities that may have affected the health of the foetus. We have found a large commitment to altruism amongst pregnant women in donating placentae for research [19].

BeWo B30 Clone Monolayer Cell Transfer Model

The BeWo cell line is an immortalized trophoblastic cell line of human origin which has proven useful in transport studies because the cells form polarized, confluent monolayers [7]. This choriocarcinoma cell line serves as an *in vitro* model of the rate-limiting barrier to maternal–foetal exchange, which has been used not only in a variety of transport studies, but also to investigate placental metabolism [9]. The BeWo b30 model consists predominantly of cytotrophoblast cells which form a confluent monolayer with tight junctions, but they do not spontaneously differentiate to syncytiotrophoblast, and the model lacks connective tissue and foetal endothelium which are present in the intact human placenta. We used the cell culture protocol of Bode *et al.* [7], and the BeWo monolayer transport model was adapted from the protocol described in Poulsen *et al.* [20]. In brief, cells are cultured in DMEM-F12 (Sigma-Aldrich, Ayrshire, UK) containing 10% FBS (In vitro, Copenhagen, Denmark), penicillin/streptomycin and glutamine. They are maintained at 37°C, 5% CO_2 and 95% relative humidity. Cells are passaged every 4–5 days, and passage number is noted for each study. The BeWo clone b30 cells were provided by Dr. Margaret Saunders (Bristol Haematology and Oncology Centre) with permission from Dr. Alan Schwartz (Washington University, St. Louis, MO, USA).

For transport studies (fig. 2), cells are seeded in polyester/polycarbonate Transwell® inserts (pore size 0.4 or 3 μm, 1.12 cm^2 growth area, apical volume 0.5 mL, basal volume 1.5 mL, Corning Costar, New York, NY, USA) non-coated or coated with human placental collagen (Sigma-Aldrich, St. Louis, MO, USA), at a cell density of 100,000 cells/cm^2. The medium is changed daily after seeding until the cell monolayers reach confluency (approximately 5 days), which is monitored by visual inspection using light microscopy. Transepithelial electrical resistance (TEER) is measured using an EndOhm apparatus (World Precision Instruments, Sarasota, FL, USA) at room temperature. Only inserts with cell monolayers that show TEER values above 35 Ω cm^2 are used for transport studies. Before adding the substance of interest to the apical (maternal) chamber, the cells are equilibrated in transport medium, which is DMEM-F12 without phenol red, in both apical and basal chambers for 30–45 min at 37°C.

The transport experiments are performed under cell culture conditions with constant shaking (30 rpm) on an orbital shaker. At time 0, the test substance is added to the apical chamber, and samples of 5 and 100 μL are taken from the apical (maternal) and basal (foetal) chambers, respectively, at the designated time intervals. The 100 μL sample taken from the basal chamber is replaced by fresh transport medium. The 5 μL sample from the apical chamber is not replaced. After the final time-point, the cells are washed three times with ice-cold Hank's Balanced Salt solution (Sigma-Aldrich) and the Transwell® membranes are removed and lysed overnight at 4°C in lysing solution (0.5% Triton X-100 in 0.2 N NaOH). Experiments are carried out using 5–6 inserts per compound (plus three blanks).

Comparison of the Placental Perfusion Model and the BeWo Cell Transfer Model

The major differences in predicting placental transfer by using the placental perfusion model, the BeWo model and maternal/foetal blood ratio are illustrated in table 1. The transport of substances across placenta in the placental perfusion model has a higher rate compared to that across the BeWo cell monolayer, due to the pressure and flow in the circulation pump set-up. The BeWo cell monolayer model is placed on a shaking plate to create flow, but this flow across the cytotrophoblast layer is not in the same magnitude as the placental perfusion model, and there is no hydrostatic pressure on the foetal side in the BeWo model as there is in the perfusion model. However, it is still possible to compare transport

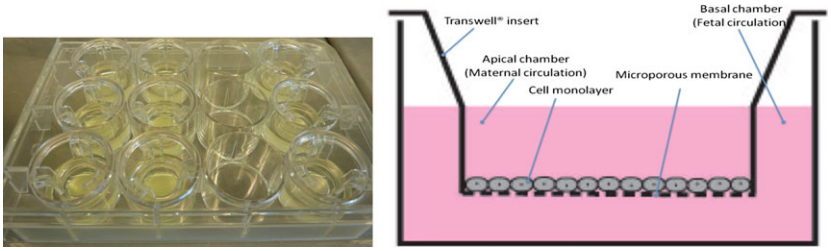

Fig. 2. BeWo cell transport assay. Left: 9 Transwell® inserts in a 12-well plate. Right: Illustration of one Transwell® insert with a monolayer of BeWo cells seeded on the permeable membrane (figures provided with permission by Marie Sønnegaard Poulsen).

kinetics within each model, and several substances can be ranked in the order of placental transport by studying the kinetics of transport in the two systems. This is described in Poulsen *et al.* [20] where caffeine, benzoic acid and glyphosate, including the positive control substance antipyrine, which were previously studied in the placental perfusion model [21], were also studied in the BeWo cell monolayer transfer model. The substance transport kinetics of the two models ranked similarly, suggesting transferability between results from the two models. The initial slope of the transfer of substance was approximately ten times higher in the placental perfusion model compared to the transfer in the BeWo cell model, and the time to equilibrium observed for perfusion experiments was ten times longer in the BeWo cell model. Nevertheless, the shapes of the transport curves were very similar for placental perfusion and BeWo cell monolayer transfer of the same compound, although with different time scales. Furthermore, a recent investigation by Li *et al.* [22] expanded this comparison with nine compounds; they observed excellent correlation of BeWo cell transport data and perfusion transfer indices ($R^2 = 0.95$).

Because of the comparability of results from the placental perfusion model with the BeWo cell model, the BeWo cell monolayer model can be used as a first-step transfer model when investigating the placental transfer of a given substance. BeWo cell transfer results may suffice if they are found to be consistent with the expected based on substance properties [20], but if more complete information is preferred, further investigation with the more complex *ex vivo* placental perfusion model is recommended [23]. The purpose of the study can also affect the choice of the model, as the placental perfusion model reflects interindividual differences in transport regarding receptors and metabolism, whereas the BeWo cell line is derived from one individual and gives a more homogenous result. In the case of investigating drugs or environmental substances, there is the possibility of comparing blood samples from the mother at the time of delivery and umbilical cord blood. The concentration ratio of these samples will give a true measure of placental transport and foetal exposure, though it is only possible for substances with maternal exposure at the time of delivery, or for substances with a very long half-life/constant exposure.

The advantages of the BeWo cell model are the relatively simple set-up and execution of the experiments, as opposed to the placental perfusion model, which is dependent on the placental quality and is technically more challenging. Nevertheless, the BeWo cells may be susceptible to certain toxic agents. For example, in our study of placental transfer of Benzo[α]pyrene (BaP), the BeWo monolayer model could not uphold a viable monolayer due to toxicity of either BaP or the solvent toluene in the same concentration that was used in the placental perfusions. While *in vitro* studies using BeWo cells

Table 1.
Comparison of experimental models for the determination of maternal-to-foetal transfer.

Model	Placental perfusion	BeWo	Maternal/foetal blood ratio
Study tissue	Human placental tissue	Derived from human choriocarcinoma	Human blood samples
	Multi-nucleated syncytiotrophoblast	Undifferentiated single nucleated trophoblasts	Plasma or serum
Representation	Complete tissue	Trophoblast cells only	Real exposure situation
	Multi-nucleated syncytiotrophoblast	Undifferentiated single nucleated trophoblasts	Plasma or serum
Extrapolation considerations	Placentas delivered at term	Characteristics from different gestational time-points	Samples collected at birth
	Interindividual heterogeneity	Originates from one person	Interindividual variation
Kinetic driver	Pump generated flow creating pressure	Stir plate imitating flow – no pressure	Physiological transport
Equilibrium	Antipyrine equilibrium after approximately 90 min	Antipyrine equilibrium after approximately 12 hr	Equilibrium at birth
Exposure period	2.5- to 6-hr experiments	3- to 24-hr experiments	Full pregnancy
Test compound	100 mL test compound per experiment	5 mL test compound per experiment	Not relevant
Experimental success	Low experimental success rate	High experimental success rate	High success
Ethical considerations	Informed consent from mother needed	No consent needed	Informed consent from mother needed

are complementary to placental perfusion experiments, they cannot replicate the *in vivo* similarity inherent to the more sophisticated placental perfusion model. Rather, the BeWo cell model can be used as a screening tool to optimize the selection of experiments to be pursued in greater detail using the *ex vivo* placental perfusion model.

Placental Transport of Molecules

A range of substances have been studied in the placental perfusion model in Copenhagen. The perfusion kinetics are described in table 2. The characteristics determining the placental transport kinetics are:

- Physicochemical properties such as size and lipophilicity.
- Adsorption to the placental tissue, preventing transport to the foetus.
- Binding to transport proteins such as albumin, facilitating or preventing transport.
- Binding to receptor proteins on the placental membranes, facilitating or preventing feto-maternal transfer.
- Metabolism of the substance by the placenta, creating a new substance with different properties.

The *ex vivo* placental perfusion system was used to study the placental passage of thirteen dietary and environmental compounds investigated for foetal exposure [24]: ^{14}C-benzo[a]pyrene (BaP), ^{14}C-2-amino-3-methyl-imidazol[4,5-f]-quinoline (IQ), ^{14}C-2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhIP), acrylamide (AA), glycidamide (GA), ^{14}C-nitrosodimeth-

Table 2.

Placental transfer kinetics of substances studied in Copenhagen.

Substance	Placental transfer kinetics
Placental perfusion model	
Benzo(α)pyrene (BaP) Product of incomplete combustion. BaP is the most studied carcinogenic PAH and is often used as a toxicological prototype or surrogate for all carcinogenic PAHs.	BaP has previously been studied in a guinea pig placental perfusion model [33,34] and in human umbilical cord blood samples *in vivo* [35,36]. These studies show that BaP has a slow transport across the placenta, but that it crosses both in its metabolized and un-metabolized form. BaP is bound by albumin, and our studies demonstrate a correlation between the albumin media concentration and the transport of BaP across the placenta, indicating albumin as an important transport molecule. Albumin does not cross the placental barrier, but it is taken up by trophoblast cells and recycled to the maternal compartment [37]. BaP is activated by the phase 1 enzyme CYP1A1, which is present in the placenta. Concentration of the water-soluble metabolite was increasing throughout the six-hour placental perfusion [28].
Polybrominated diphenyl ethers (PBDEs) Flame retardants in various consumer products. PBDEs may affect thyroid hormone homeostasis.	BDE-47 was transported across the placenta to a greater extent than BDE-99. The compound transport may be affected by molecular size and resulting steric hindrance or possibly differences in affinity for transport proteins as, for example, albumin which may control the transport of the larger congeners. The majority of BDE-47 and BDE-99 was adsorbed to the placental tissue.[38].
Placental perfusion model and BeWo cell model	
Polychlorinated biphenyls (PCBs) Persistant and bioaccumulating. Human population is still exposed to PCBs mainly via animal based foods such as meat, dairy and fish.	Placental transport of two non-dioxin-like PCBs (PCB52 and 180) were studied in the Copenhagen placental perfusion model and in the BeWo b30 clone monolayer transfer model in Bristol [39]. Both PCBs transferred rapidly from the maternal into the foetal circulation, PCB180 crossing the placental barrier faster than PCB52. Recovery rates for both PCBs at the end of the perfusion were below 50%. PCB52 adsorbed more to the tissue of the perfused cotyledon than ^{14}C-PCB180. Both the FM ratio and permeability rate were about three times higher in the perfusion model than in the BeWo model. The recovery of ^{14}C-PCB from the *ex vivo* system was lower than that from the *in vitro* model.
Nanoparticles Fluorescent polystyrene nanoparticles, (50 nm, 100 nm), silica nanoparticles (25 nm, 50 nm)	Good agreement has been noted for nanoparticles studied using both the BeWo cell experiments and the placental perfusions. Size-dependent transport of polystyrene nanoparticles was evident in both models [40,41], and the transfer of silica nanoparticles was quite limited in both types of experiments [42].
Recombinant antibodies Two human IgG3 antibodies with a hinge deletion (IgG3ΔHinge) that eliminate complement activation and Fcγ receptors (FcγRs) and C1q binding, but binding to FcRn is retained. One antibody has a single point mutation in the Fc (R435H) at the binding site for FcRn (IgG3ΔHinge:R435H).	We compared transplacental transport with wild-type IgG1 and IgG3 and found transport across trophoblast-derived BeWo cells and *ex vivo* placenta perfusions with hierarchies as follows: Placental perfusion:IgG3ΔHinge:R435H ≥ wild-type IgG1 ≥ IgG3ΔHinge BeWo transfer model: IgG3ΔHinge:R435H = wild-type IgG1 = wild-type IgG3 >>> IgG3ΔHinge The placenta perfusion model showed very impaired transport of the recombinant antibody with hinge deletion (IgG3ΔHinge), demonstrating receptor-dependent transport, whereas the BeWo cell transfer model transported the molecule with transport kinetics comparable to the wild-type IgG1 [32].

ylamine (NDMA), aflatoxin B1 (AFB1), deoxynivalenol (DON), ^{14}C-2,20,5,50-Tetrachlorobiphenyl (PCB52), ^{14}C-2,20,3,4,40,5,50-Heptachlorobiphenyl (PCB180), ^{14}C-bisphenol A (BPA), 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD) and ethanol (EtOH). Three placental perfusion laboratories were involved in the perfusions, and commonly agreed protocols generated within the European research programmes of ReProTect (Development of a novel approach in hazard and risk assessment of reproductive toxicity by a combination and application of *in vitro*, tissue and sensor technologies) and NewGeneris were used [23]. Perfusion transport data of investigated chemicals were compared and categorized according to their ability to pass the placental barrier.

Due to the differences in the perfusion flow, interlaboratory differences and cotyledon differences, a meta-analysis with normalization of data to antipyrine transfer was performed on all data. The data variance was reduced, suggesting that at least some of the perfusion-to-perfusion variation, for example, due to differential flow conditions was reduced. Antipyrine normalization resulted in the following rank order of compounds according to their transfer values: NDMA ≥ EtOH ≥ BPA ≥ IQ ≥AA ≥ GA ≥ PCB180 ≥ PhIP ≥ AFB1 > DON ≥ BaP ≥ PCB52 ≥ TCDD [25].

Normalization generated a clear division of the studied compounds into fast, medium and slow transfer groups. Compounds with a small molecular mass (500 Da), low polarity, moderate lipid solubility and low protein binding capacity generally cross the placenta by passive diffusion. The partition coefficient between octanol and water (log K_{ow}) and the dissociation pH (pK_a) may also predict transplacental behaviour, provided the compound crosses the placenta by passive diffusion, because the passage of hydrophilic highly ionized compounds is limited by the lipid membranes of the placenta [26,27]. The transplacental transfer rate can also be affected by compound binding to plasma proteins, for example the transport was increased for both BaP [28] and BPA [29]. Also, the differences in binding capacity of maternal and foetal albumin may affect the *in vivo* placental transfer. Amongst a number of compounds having molecular masses below 500 Da and being non-ionized at physiological pH, we found the placental transfer to rank from fast and facile (e.g., NDMA) to very limited transfer (TCDD). The compounds having transfer rates very similar to simple diffusion are NDMA, EtOH, BPA, AA, GA and IQ, of which NDMA, EtOH, AA and GA have small molecular masses and are soluble in water. PhIP and AFB1 can bind to the ABCG2 efflux transporter located in maternal facing syncytiotrophoblast, which may prevent the facile entry of these chemicals into the foetal compartment [30]. Interestingly, IQ is also a substrate for ABCG2, but it seems that in human placenta ABCG2 does not limit its transfer [31].

DON, BP, PCB52 and TCDD are compounds with a slow transplacental transfer rate. The limited transfer of DON may be due to its relatively high ability to form hydrogen bonds to water or that DON is a substrate for an efflux transporter.

Good agreement was found in the transfer kinetics of all substances studied in both the placental perfusion model and the BeWo cell model, except in the study of recombinant antibodies. This may be due to transfer kinetics of foetal endothelium, which is intact in the placental perfusion model, but not present in the BeWo transfer model, or the competitive inhibition of endogenous antibodies on the FcRn-receptor in the placental perfusion model [32].

The placenta can act as a sink and metabolizing organ throughout pregnancy, protecting the foetus but also exposing the foetus to environmental toxicants and their metabolites. When toxicants adsorb to the placental tissue, the foetus is protected from these toxicants; however, if the toxicants compromise the function of the placenta, the foetus will still be affected, for example, by hypoxia that may be followed by pre-eclampsia. Nanoparticles may also affect the placental balance by disruption of the barrier and by oxidative stress as indicated by the use of biomarkers of oxidative stress.

Perspectives

Ex vivo dual placental perfusion is often used to study placental physiology in normal and abnormal placental tissue. With further adaptation of the model, *ex vivo* dual perfusion may be used as a disease model. We have implemented the placental perfusion model to study placental malaria. Preliminary results show that adhesion of malaria parasites can be studied in the model, and we are currently investigating whether the model can be used to evaluate potential placental malaria vaccine constructs. The model may also be used to study the pathophysiology of placental malaria, including placental inflammatory response and coagulation, gene expression and transplacental transport of macromolecules such as nutrients and IgG.

The placental perfusion model could be a useful tool in investigating the interaction between the hFcRn receptor and other molecules, for example, related to malaria. This takes the human placental perfusion model further than just being a tool for foetal risk assessment. There is a genuine opportunity of investigating the physiology of human receptors and metabolism in a whole organ, *ex vivo*.

Conclusion

Our primary goal is the use of human tissue for human risk assessment of foetal exposure to environmental toxicants. The optimal and most detailed results are accomplished by combining the relatively simple monolayer culture system of human BeWo cells with the *ex vivo* human placental perfusion system and possibly *in vivo* blood sampling. Substances of interest can be categorized according to their transfer rate across the two models. The limitations of the monolayered culture compared to the three *in vivo* barriers separating the maternal and foetal circulations must of course be taken into account. Thus, we recommend initiating studies of placental transport with the BeWo cells to estimate levels of toxicity, binding and the role of transporters. For any conclusive statements, transport data from the

perfusion system and/or concentration measurements of compounds in umbilical cord blood and maternal blood at birth must be added.

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