Toxicology in Vitro 23 (2009) 1380–1386

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Modeling placental transport: Correlation of *in vitro* BeWo cell permeability and *ex vivo* human placental perfusion

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ARTICLE INFO

Article history:
Received 16 June 2009
Accepted 24 July 2009
Available online 30 July 2009

Keywords:
Placental transport
BeWo cells
Modelling
Caffeine
Benzoic acid
Glyphosate

ABSTRACT

The placental passage of three compounds with different physicochemical properties was recently investigated in *ex vivo* human placental perfusion experiments (caffeine, benzoic acid, and glyphosate) [Mose, T., Kjaerstad, M.B., Mathiesen, L., Nielsen, J.B., Edelfors, S., Knudsen, L.E., 2008. Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. J. Toxicol. Environ. Health, Part A 71, 984–991]. In this work, the transport of these same three compounds, plus the reference compound antipyrine, was investigated using BeWo (b30) cell monolayers. Transport across the BeWo cells was observed in the rank order of caffeine > antipyrine > benzoic acid > glyphosate in terms of both the apparent permeability coefficient and the initial slope, defined as the linear rate of substance transferred to the fetal compartment as percent per time, a parameter used to compare the two experimental models. The results from the *in vitro* studies were in excellent agreement with the *ex vivo* results (caffeine ≈ antipyrine > benzoic acid > glyphosate). However the transfer rate was much slower in the BeWo cells compared to the perfusion system. The advantages and limitations of each model are discussed in order to assist in the preparation, prediction, and performance of future studies of maternal–fetal transfer.

1. Introduction

Understanding the placental transport of compounds provided to the pregnant mother from the environment, lifestyle or by medication is essential to reduce the risks of fetal exposure to harmful substances during pregnancy. The importance of protecting the developing fetus from possible adverse effects in development and growth has been especially salient since it was recognized in the 1960s that the administration of thalidomide during pregnancy causes severe birth defects (Yaffe, 1998). Today, special attention is also placed on environmental chemicals that may prenatally disturb the development of the reproductive system by endocrine disruption (Mahood et al., 2007).

The placenta serves as the interface between the maternal and fetal circulations during pregnancy. Fetal blood vessels from the umbilical cord branch out to form villous trees within the placenta in spaces called placental lobes, or cotyledons. The villous trees are bathed in maternal blood, and only three layers separate maternal and fetal blood in the mature placenta: (1) syncytiotrophoblast cells, (2) a thin layer of connective tissue, and (3) the fetal vascular endothelium (Sastry, 1998). Syncytiotrophoblast cells are multinucleated cells formed from the fusion of the precursor cytotrophoblast cells, and it is within this layer that the transport of nutrients, elimination of fetal waste, and other processes related to maintaining the pregnancy are regulated (Audus, 1999). It is not until the third month of pregnancy that the placenta is fully differentiated (Sastry, 1998).

Transport of molecules – be they nutrients or other endogenous compounds, drugs, or xenobiotics – across the placental barrier depends upon physicochemical properties such as size, charge, and lipophilicity. In general, compounds with appropriate lipophilicity (usually characterized by the octanol–water partition coefficient, log *P*) can passively diffuse across cells. This is referred to as transcellular transport. Small, hydrophilic compounds may be able to pass in between the tight junctions of cells, which is called paracellular transport. There are several exceptions to these general statements, however, including compounds recognized by active in- or efflux transporters; endocytosis is a slower process than the aforementioned mechanisms, but it is significant in the transport of certain substances, such as antibodies (Ellinger et al., 1999). Other features of the placental barrier also play a role in the transplacental passage of chemical compounds, including a thinner syncytiotrophoblast layer and more surface area as gestation progresses, a small hydrostatic gradient towards the fetal compartment, and a pH gradient, with the fetal side being slightly more acidic (Audus, 1999).

In order to reduce the risk of adverse effects upon the health and viability of the developing fetus, the concentration of xenobiotics in the fetal circulation must be minimized, including

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doi:10.1016/j.tiv.2009.07.028

environmental toxicants and any drugs used to treat a medical condition in the mother. Some women have chronic conditions during pregnancy that require treatment, including diabetes, asthma, and epilepsy (Audus, 1999). In addition, illnesses may arise during pregnancy, and maintaining the health of the mother is an integral part of maintaining the health of the developing fetus. Most drugs will cross the placenta to some extent unless they are destroyed or altered by metabolism or other factors that limit their transport across the placental barrier (Yaffe, 1998). Protective mechanisms in the placental barrier, such as the P-glycoprotein efflux pump, only reduce the transport of certain molecules, rather than prevent their transport (Audus, 1999).

As mentioned above, the syncytiotrophoblast cell layer serves as the rate-limiting barrier for the exchange of compounds between the maternal and fetal circulations. Experimental models used to study placental drug transport should replicate as much as possible the full features and functionality of trophoblast cells *in vivo*. For obvious ethical reasons, *in vivo* experimentation of placental drug transport in humans is not feasible on a large scale. *In vivo* experimentation in animal models provides the advantages of a complete physiological system, but the placental structures found in other species do not match the unique hemomonochorial, multivillous nature of the human placenta (Moe, 1995). For example, in the placentae of rabbit and guinea pig, a single layer separates maternal and fetal blood, but there are five layers in the syndesmochorial placenta of sheep (Rudolph, 1995). Interspecies differences in placental morphology and length of gestation necessitate the use of caution in assuming that the maternal-to-fetal transfer of substances observed in animal models will be equally observed in humans (Enders and Blankenship, 1999). For these reasons, mechanisms of placental transport, metabolism, and placental toxicity are best investigated in models of human origin (Myllynen and Vahakangas, 2002).

Several strategies have been utilized to study placental drug transport *in vitro*. Examples include villous explants (Bechi et al., 2006), perfused placental lobes, isolated membrane vesicles, primary cultured cytotrophoblast cells, and cell lines (Moe, 1995). The dually perfused placental cotyledon is an *ex vivo* method which has proven useful for clearance and transport studies, and placental perfusion experiments generally show good correlation with *in vivo* data (Myllynen and Vahakangas, 2002).

Ex vivo placental perfusion provides a unique opportunity to carry out valuable research without ethical difficulties; the experiments are non-invasive and do not interfere with the care of the mother or newborn child (Myren et al., 2007). Although placental function *in vivo* is no longer required after delivery, perfusion conditions allow for continued placental tissue viability for several hours (Di et al., 2003). It takes around 30 min following the birth to set up a perfusion, but the increased tolerance to hypoxia and recovery from the ischemic period upon reperfusion of the placenta is suggested to be due to energy saving mechanisms and downregulated metabolism, similar to what is observed in hibernating mammals or deep sea diving turtles (Schneider, 2009). Placentae from elective caesarean sections are more likely to display reduced oxidative stress and molecular conditions more similar to the *in vivo* state compared to placentas subjected to labor (Cindrova-Davies et al., 2007). Viability of the placenta during the experiments is verified by monitoring leakage from the fetal compartment, oxygen transfer, and glucose consumption; appropriate antipyrine transfer between the maternal and fetal circulations confirms proper experimental set up and can be used to normalize differences between placentas (Vahakangas and Myllynen, 2006). Other advantages of placental perfusion experiments compared to some other models include the retention of *in vivo* placental organization and assessment of binding to placental tissue (Myllynen et al., 2005).

In addition to transport studies, placental perfusion experiments can also provide information regarding the metabolism of substances by placental enzymes. For example, the metabolism of 17-α-hydroxyprogesterone caproate was followed during perfused placental experiments, and the major metabolite was found in both maternal and fetal circuits (Hemauer et al., 2008). Furthermore, the placental conversion of the pro-carcinogen benzo[α]pyrene to water-soluble metabolites has also been observed during *ex vivo* placental perfusion (Mathiesen et al., 2009). The influences of efflux transport mechanisms upon transplacental transfer has also been studied with the dually perfused term placenta. Fetal exposure to 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was increased in the presence of KO143, an inhibitor of ABCG2 (BCRP), and using BeWo cells (ATCC clone), the expression of ABCG2 mRNA was shown to be induced by PhIP (Myllynen et al., 2008). After demonstrating the appearance of beta-lactoglobulin in the fetal side of a perfused cotyledon (Szepfalusi et al., 2000), the transport of beta-lactoglobulin was also observed in BeWo cells (clone b24) (Szepfalusi et al., 2006).

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 (Mitra and Audus, 2008). It should be noted that the b24 and b30 BeWo clones developed by Dr. Alan Schwartz have demonstrated this monolayer-forming ability which is not evident in the original BeWo clone available from the American Type Culture Collection (ATCC) (Bode et al., 2006). This choriocarcinoma cell line serves as an *in vitro* model of the rate-limiting barrier to maternal–fetal exchange (Rytting and Audus, 2008) which has been used not only in a variety of transport studies (Bode et al., 2006), but also to investigate placental metabolism (Avery et al., 2003). The BeWo b30 model consists predominantly of cytotrophoblast cells which form a confluent monolayer with tight junctions, but they do not spontaneously differentiate to syncytiotrophoblasts, and the model lacks the connective tissue which is present *in vivo* and in the perfused placenta.

The placental passage of three compounds with different physicochemical properties was recently investigated in *ex vivo* human placental perfusion experiments. Caffeine displayed the highest rate of transfer from maternal to fetal perfusate, benzoic acid transport reached a steady-state level after an initially limited transfer rate, and the transport of glyphosate was much lower (Mose et al., 2008). In this work, the transport of these same three compounds, plus the reference compound antipyrine, is investigated using BeWo cell monolayers. The data obtained from both types of experiments are compared and the advantages and limitations of each model are discussed in order to assist in the preparation, prediction, and performance of future studies of maternal–fetal transfer.

2. Methods

BeWo cells (clone b30) were obtained from Dr. Margaret Saunders (Bristol Haematology and Oncology Centre) with permission from Dr. Alan Schwartz. Cell culture was carried out following previously described protocols (Bode et al., 2006). Briefly, the cells were cultured in DMEM (Sigma–Aldrich, Ayrshire, UK) with 10% FBS (In vitro, Copenhagen, Denmark) supplemented with penicillin/streptomycin (Panum Institute, University of Copenhagen) and glutamine (In vitro, Copenhagen, Denmark) and maintained at 37 °C, 5% CO_2, and 95% relative humidity. Cells were passaged every 4–5 days; cell passage numbers 20–43 were used in this study. Prior to transport experiments, cells were seeded at a density of 100,000 cells/cm^2 onto polyester Transwell® inserts (pore size 0.4 μm, 1.12 cm^2 growth area, apical volume 0.5 mL,

basolateral volume 1.5 mL, Corning Costar, New York, USA) coated with human placental collagen (Sigma–Aldrich, St. Louis, USA). The cell culture medium was changed daily until the cell monolayers reached confluency (after 5–6 days) which was monitored by visual inspection with light microscopy.

The formation of tight junctions for barrier integrity was also investigated by measuring the trans-epithelial electrical resistance (TEER) using an EndOhm apparatus (World Precision Instruments, Sarasota, FL). The TEER was measured in growth media (DMEM) at room temperature and calculated as the measured resistance minus the resistance of an empty Transwell (blank without cells). Barrier functionality was confirmed by comparing the TEER values of the cell layers at several time points post-seeding (days 3–7) to the monolayer confluency and the transport of sodium fluorescein (Fluka Analytical/Sigma–Aldrich, St. Louis, USA) and FITC-Dextran (FD-40, Sigma, St. Louis, MO) and because of these results, only the Transwell inserts with cell monolayers displaying TEER values between 35 and 60 $\Omega\,cm^2$ were used. According to the observations by Liu et al. (1997), this resistance indicates the presence of a single monolayer on the filters. Following the TEER measurements, the cells were equilibrated in transport medium (DMEM without phenol red) in both apical and basolateral chamber for 30–45 min at 37 °C. At time 0, the compound of interest dissolved in transport medium was added to the apical chamber. The transport experiment was carried out under cell culture conditions (37 °C, 5% CO_2, 95% humidity) with constant stirring (70 rpm). At the specified time points, 100 μL samples were removed from the basolateral chamber and replaced with 100 μL of fresh transport medium. At the final time point, a 100 μL sample was also removed from the apical chamber, and the cells were washed three times with ice-cold HBSS (Sigma–Aldrich, Ayrshire, UK). The Transwell inserts were then removed and the cell monolayer was lysed for 2 h with stirring at 37 °C in lysing solution (0.5% Triton X-100 in 0.2 N NaOH). Experiments were carried out in 4–6 Transwells per compound (plus 3 blanks). The transport of caffeine (ARC-0235 Caffeine, [1-methyl-14C] American Radiolabeled Chemicals Inc., St. Louis, USA and ACROS Organics, NJ, USA), benzoic acid (ARC-0187 Benzoic acid [ring-14C(U)], American Radiolabeled Chemicals Inc., St. Louis, USA and ACROS Organics, NJ, USA), and glyphosate (ARC-1312 Glyphosate, [glycine 2-14C] American Radiolabeled Chemicals Inc. (ARC), St. Louis, USA and Fluka Analytical/Sigma–Aldrich, St. Louis, USA) were studied at a concentration of 200 μM. The specific activity of each was 1 μCi/mmol, diluted from unlabeled and labeled caffeine (0.1 mCi/mL, 55 mCi/mmol), benzoic acid (0.1 mCi/mL, 60 mCi/mmol) and glyphosate (0.05 mCi/mL, 55 mCi/mmol) respectively. The transport of antipyrine (Aldrich-Chemie, Steinheim, Germany) was investigated at a concentration of 100 μg/mL. Concentrations of caffeine, benzoic acid, and glyphosate in transport medium and in cell lysate were determined using liquid scintillation counting (Perkin–Elmer Tri-Carb 2800TR, Waltham, Massachusetts) together with standards and background samples. Antipyrine was analyzed by HPLC (LaChrom high-performance Liquid Chromatography system equipped with a C-18 column (Macherey-Nagel GmBH & Co., Düren, Germany) and a SecurityGuard precolumn (ODS, Octadecyl), mobile phase 45% methanol (Sigma–Aldrich, St. Louis, MO), flow rate 1 mL/min, detection at 254 nm) with external and internal standards. Cellular uptake data were corrected for protein content, which was determined from the cell lysate using a BCA kit (Pierce).

For the samples at each time point $t = t_n$, the mass transported (ΔQ_n) was determined and corrected for the mass removed during the previous sampling periods using the following equation:

$$\Delta Q_n = C_n \cdot V_w + \sum_{j=1}^{n-1} V_s \cdot C_j \quad (1)$$

where C_n is the concentration of the sample measured at time t_n, V_w is the volume of the well sampled (in this case, 1.5 mL from the basolateral side of 12-well Transwells), V_s is the sampling volume (in this case, 100 μL), and the term $\sum_{j=1}^{n-1} V_s \cdot C_j$ represents the correction for the cumulative mass removed by sampling during all the sampling periods prior to t_n (from $t = t_1$ until $t = t_{n-1}$).

These data were then converted to permeability values to correct for the transport of each substance across empty Transwell filters. Permeability (P) is calculated with the following equation:

$$P = \frac{\Delta Q/\Delta t}{A \cdot C_0} \quad (2)$$

where $\Delta Q/\Delta t$ is the rate of the substance flux across a layer (mass/s), A is the surface area of the layer (in cm^2), and C_0 is the initial concentration of the substance on the donor side (in mass/cm^3). The permeability (P) of the substance is then expressed as cm/s. In order to calculate the permeability across the cell monolayer alone (and to correct for the influence of the coated membrane on permeability), transport of the substance across blank filters (the coated Transwell membrane alone without cells) was measured. The permeability in these blank experiments (P_m) and the permeability in the experiments with cells (P_t) can then be used in the following equation derived from Utoguchi et al. (1999) to calculate the apparent permeability across the cell monolayer alone (P_e):

$$P_e = \frac{1}{\left(\frac{1}{P_t} - \frac{1}{P_m}\right)} \quad (3)$$

3. Results

The confluency and barrier integrity of the BeWo cell monolayer was verified by visual examination (light microscopy) and trans-epithelial electrical resistance (TEER). When the cell culture medium was changed daily, the TEER increased gradually, with a value of 51.2 ± 5.4 $\Omega\,cm^2$ at day 6. Changing the cell culture medium of the BeWo cells in Transwell plates every other day, on the other hand, resulted in alternating decreases and increases in TEER values. The TEER values (when changing the cell culture medium daily) were further correlated to the transport of sodium fluorescein and FITC-Dextran (FD-40) The permeability of both fluorescein and FD-40 decreased with increasing TEER, indicating an increase in the integrity of the monolayer in the first 5–6 days post-seeding. Fig. 1 correlates fluorescein transport with TEER for days 3–7 post-seeding. FITC-Dextran transport across the BeWo cell monolayer is lower than the fluorescein transport due to the larger size of the molecule, but the correlation with the TEER values is comparable to that observed with fluorescein. Based on these results, the BeWo cell transport experiments described below were performed on days 5 or 6 post-seeding.

Fig. 2 shows the transport of caffeine, antipyrine, benzoic acid, and glyphosate across BeWo cell monolayers in the apical to basolateral direction as well as their transport across blank Transwell inserts (without any cells). When BeWo cells grow on collagen-coated Transwell inserts, they polarize such that the apical chamber represents the maternal side of the placental barrier (Bode et al., 2006). In terms of the percentage of substance which crossed to the fetal side, the transport of all four compounds across blank inserts was similar. The transport across the trophoblast cell layer was fastest for caffeine and antipyrine. In fact, the passage of caffeine across the cells was almost as fast as its passage without cells, indicating a high degree of unrestricted diffusion for caffeine. The diffusion of antipyrine, which is used as a control parameter in *ex vivo* placental perfusions, was also rapid. Benzoic acid displayed an intermediate degree of transport across BeWo cell monolayers, and glyphosate transport was the slowest.

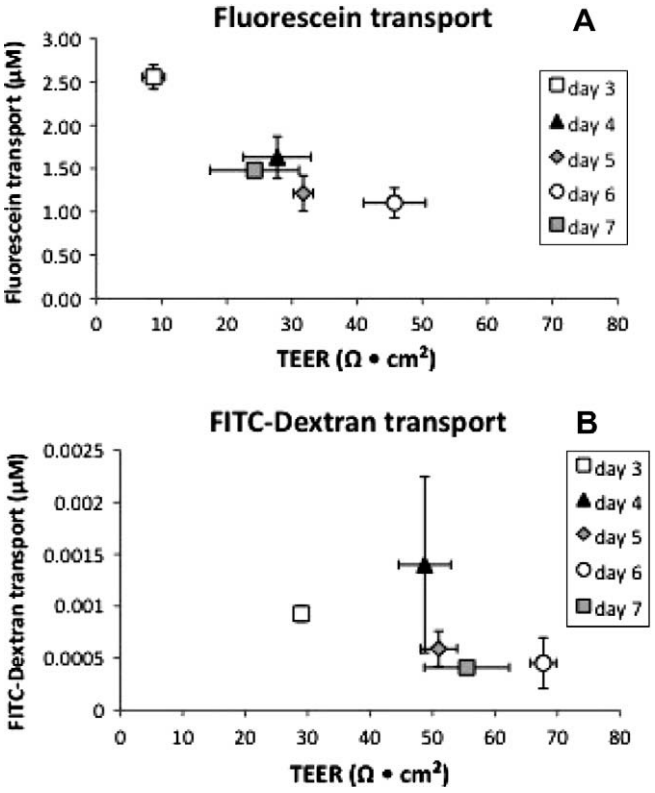

Fig. 1. Correlation of trans-epithelial electrical resistance (TEER) and sodium fluorescein (A), FITC-Dextran (B) transport across BeWo cell monolayers at different time points post-seeding in 12-well transwell inserts. The fluorescein and FITC-Dextran transport is presented as the concentration of fluorescein reaching the basolateral (fetal) 2 compartment 30 min after dosing 100 µM fluorescein (A) and 5 µM FITC-Dextran (B) to the apical (maternal) chamber.

The transport of these four compounds from the maternal to the fetal compartment in *ex vivo* placental perfusion experiments was reported previously (Mose et al., 2008). Fig. 3 compares the transport of caffeine, antipyrine, benzoic acid, and glyphosate from the present *in vitro* BeWo cell experiments to their reported transport in the *ex vivo* perfusions. Data from *ex vivo* placental perfusion experiments are usually reported with the parameter called the fetal–maternal ratio (FM ratio), which is defined as the concentration in the fetal perfusate divided by the concentration in the maternal perfusate at a given time. In contrast, transport data from BeWo cell experiments should be reported using permeability coefficients using the aforementioned equations (Eqs. (2) and (3)). Since the total cross-sectional area of the trophoblast cell layer within a perfused cotyledon is unknown in the placental perfusion experiments, it is not feasible to compute the transport from the perfusions as permeability coefficients using Eq. (2). For a more direct comparison of the data in Fig. 3, the transport of each substance is therefore presented as the percentage of the amount added to the maternal side which appears in the fetal compartment over time.

Parameters describing the transport of these four substances across BeWo cells and in placental perfusion experiments are presented in Table 1. As expected upon observation of the curves in Fig. 2 and 3, caffeine shows the highest permeability coefficient in the BeWo cell experiments and the highest initial slope (percent transported per hour). This initial slope term is calculated from the slope of the initial, linear portion of the curves in Fig. 3, using the data from the first 60 min of the BeWo cell experiments and from the first 15 min of the placental perfusion experiments. It should be noted that this is different from the indicative permeability rate defined in (Mose et al., 2008), which is calculated using the slope of the FM ratio curve rather than the percentage of compound appearing in the fetal compartment.

Table 1 shows that the compounds' transport across BeWo cells can be ranked in the order of caffeine > antipyrine > benzoic acid > glyphosate in terms of both the permeability coefficient

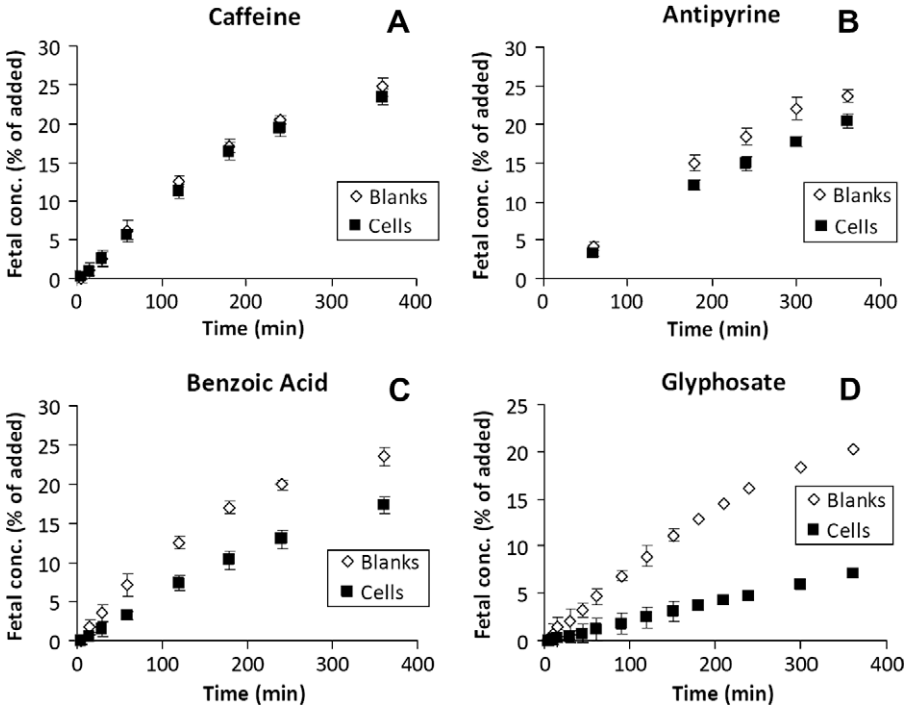

Fig. 2. Accumulation in the fetal compartment of caffeine (A, 200 µM, $n = 6$), antipyrine (B, 531 µM, $n = 6$), benzoic acid (C, 200 µM, $n = 4$), and glyphosate (D, 200 µM, $n = 6$) during transport experiments across BeWo cell monolayers at 37 °C. The transport is presented as the percentage of substance added to the maternal side at time zero and compared to the transport of each compound across blank coated Transwell inserts (without cells, $n = 3$).

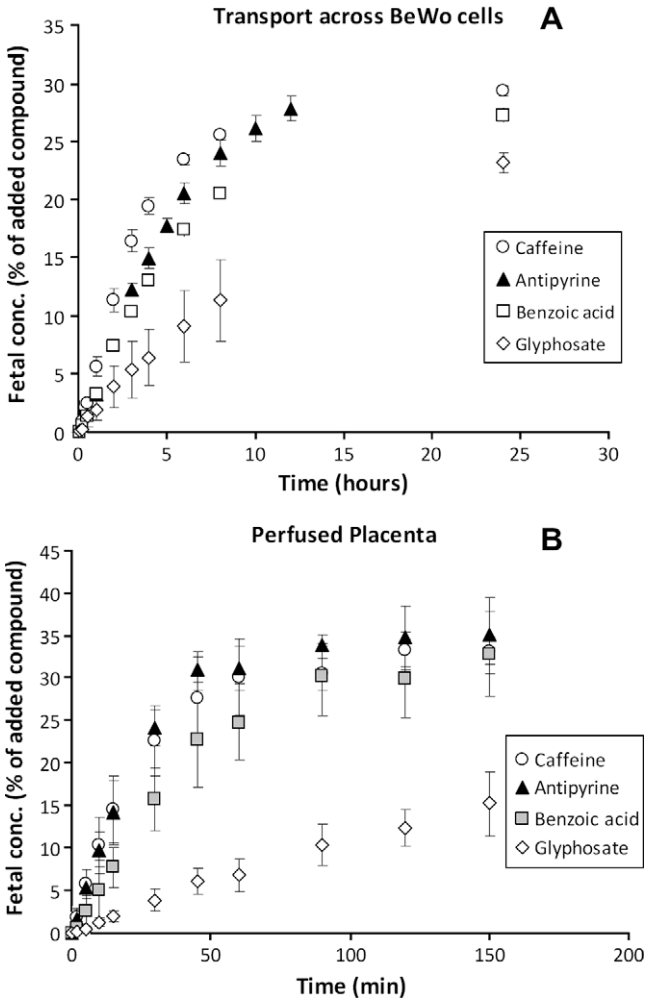

Fig. 3. Comparison of the transport of 200 μM caffeine, 531 μM antipyrine, 200 μM benzoic acid, and 200 μM glyphosate across BeWo cell monolayers (A) and in *ex vivo* placental perfusion (B). Data are presented as the percentage of substance added to the maternal side at time zero.

and the initial slope. In the *ex vivo* placental perfusions, the transport of the four compounds, using both the FM ratio and the initial slope, can be ranked in the order of caffeine ≈ antipyrine > benzoic acid > glyphosate. The recovery data appearing in Table 1 represent the mass balance in terms of the radioactivity of each compound added (except in the case of antipyrine, which was analyzed by HPLC) and removed in samples or associated with the cells or tissue at the end of the experiments. In the BeWo cell experiments, between 0.5% and 1.5% of each compound added was taken up into the cells. The total recovery calculation includes a correction for the amount of transport buffer that evaporated during the BeWo cell experiments (a rate of approximately 0.4% per hour).

4. Discussion

BeWo cell barrier function was verified prior to the described transport experiments by means of TEER measurements and assessing the permeability of fluorescein and FD-40 in agreement with what has been previously reported to verify the formation of a confluent monolayer (Liu et al., 1997). The correlation of decreased fluorescein transport with increasing TEER in Fig. 1 indicates tighter junctions as the cells reach confluency at days 5–6 post-seeding. The TEER decreases and fluorescein transport increases at day 7 post-seeding, suggesting that the optimal time for integral monolayer development has passed. The same is observed for the FITC-Dextran, but the transport is reduced compared to the fluorescein due to the larger size of the compound. These data correlated with visual inspection of cell monolayer confluency. Current protocols for BeWo cell culture call for the cell culture medium to be changed every other day (Bode et al., 2006), but since a more steady increase in TEER was observed with a daily media change, the cell culture medium for BeWo cells seeded in Transwell plates was changed every day (BeWo cells in growth flasks were still maintained with cell culture medium changes on alternating days).

This work was carried out to compare the placental transport of four compounds in both the BeWo cell line and in the dually perfused human placenta. The transport kinetics of caffeine, antipyrine, benzoic acid, and glyphosate across the BeWo cell monolayer are similar to the transport kinetics across the placental barrier in the *ex vivo* perfusion system. Antipyrine is used as a reference compound in placental perfusion experiments to monitor passive diffusion. In both models the compounds were transported in the following rank order: caffeine ≈ antipyrine > benzoic acid > glyphosate. Although Table 1 shows a higher initial slope for caffeine transport in the BeWo cell experiments compared to antipyrine, both compounds are classified as freely diffusing across the placental barrier. Benzoic acid is not transported across the placental barrier quite as quickly as caffeine and antipyrine (as seen in both the BeWo cell and perfused placenta experiments), but its transport is still substantial. The transport of benzoic acid has been

Table 1
Comparison of transplacental transport from experiments in BeWo cells and perfused human placenta.

	Antipyrine	Caffeine	Benzoic acid	Glyphosate
BeWo Cells	$n = 6$	$n = 6$	$n = 4$	$n = 6$
P_e (cm/s)[a]	$5.8 \cdot 10^{-5} \pm 2.6 \cdot 10^{-5}$	$3.2 \cdot 10^{-4} \pm 4.7 \cdot 10^{-4}$	$2.2 \cdot 10^{-5} \pm 1.8 \cdot 10^{-5}$	$5.9 \cdot 10^{-6} \pm 7.1 \cdot 10^{-7}$
Recovery (%)[b]	99.8 ± 3.1	99.9 ± 1.6	95.5 ± 2.3	102.4 ± 1.6
Initial slope (%/h)[c]	4.17 ± 0.55	5.41 ± 0.19	3.06 ± 0.09	1.12 ± 0.02
Perfused placenta	$n = 16$	$n = 4$	$n = 5$	$n = 7$
FM ratio (60 min)[d]	0.75 ± 0.12	0.79 ± 0.07	0.53 ± 0.12	0.12 ± 0.04
Recovery (%)[e]	83.3 ± 10.2	80.5 ± 8.2	80.4 ± 8.4	67.8 ± 18.5
Initial slope (%/h)[f]	60.0 ± 2.5	59.9 ± 3.2	32.8 ± 2.7	7.57 ± 0.51

[a] Apparent permeability coefficient at 60 min, as determined using Eq. (3) from the text.
[b] Mass balance of compound recovered in removed samples, remaining transport media, and cells relative to the amount initially added to the system, corrected for evaporation, determined after 24 (caffeine, benzoic acid) or 30 h (antipyrine, glyphosate).
[c] The slope of the percentage of added compound appearing in the fetal compartment in the first 60 min of the BeWo cell experiments.
[d] Fetal-maternal ratio after 60 min of perfusion (from Mose et al., 2008).
[e] Mass balance of compound recovered in removed samples, remaining perfusate, and placental tissue relative to the amount initially added to the system (from Mose et al., 2008).
[f] The slope of the percentage of added compound appearing in the fetal compartment within the first 15 min of the placental perfusion experiments.

previously reported to be mediated by a saturable carrier mechanism in both BeWo cells (Utoguchi et al., 1999) and in Caco-2 cells (Tsuji et al., 1994). The transport of glyphosate in both experimental models was much slower than the transport of the other three compounds, indicating that the placental barrier prevents a free and rapid transfer of glyphosate between the maternal and fetal circulations.

Close examination of the parameters in Table 1 reveals that although both the *in vitro* and *ex vivo* models classify the transport of these four compounds in a similar rank order, the initial slope term representing the percentage of compound transported per hour is of a much higher magnitude (approximately ten times higher) in the placental perfusion experiments compared to the initial slope calculated from the BeWo cell experiments. This is also reflected in Fig. 3, which shows that in the perfused placenta, the concentrations in the fetal compartment have already equilibrated within approximately 2 h (Fig. 3B), but in the BeWo cell experiments, no leveling off of the concentration curves is observed in the first 6 h (Fig. 3A). Further characterization of the transport of these substances across the BeWo cells over 24 h shows equilibration of the fetal concentration (Fig. 4 compares the fastest and slowest compounds), but at a time that is approximately ten times longer than the time to equilibration observed for the placental perfusion experiments. For example, the transport of antipyrine had leveled off between 10 and 12 h in the BeWo cells, and after approximately 1 h in the perfusions. This longer time to equilibration explains the order of magnitude difference in the initial slope data between the two models. Despite these differences in the time scale, the shapes of the curves are similar, there is an analogous ranking order of the compounds, and the steady-state percentage of substance transported is comparable with both models. After 2.5 h of perfusion, the percentage of compound transferred to the fetal compartment is between 30% and 35% for caffeine, antipyrine, and benzoic acid, and about 15% for glyphosate. After 24 h of BeWo cell experiments, the percentage of caffeine, antipyrine, and benzoic acid transported was near 30%, and the value for glyphosate was 24%.

The longer time required to reach an equilibrated state in the BeWo cells compared to the perfused placenta is a reflection of some differences in the two experimental procedures. The BeWo cell layer is of human trophoblastic origin, and it represents the rate-limiting barrier to placental transport. In these *in vitro* experiments, the transport buffer above and below the cell layer is rotated with a stir plate. In the *ex vivo* placental perfusions, on the other hand, maternal and fetal flow rates are controlled by peristaltic pumps. The hydrostatic gradient produced by these forced flows explains the vast difference in the resulting times to equilibration (and the subsequent initial slope data) when compared to the BeWo cell experiments, as the stirring plate cannot mimic the pressure differences present with controlled perfusion flow rates. *Ex vivo* placental perfusion is also more similar to the *in vivo* situation by virtue of the complete set of tissues comprising the placental barrier. While the BeWo cells only represent the trophoblastic cell layer, the dually perfused human placenta contains the syncytiotrophoblast cell layer as well as all connective tissue, and the fetal endothelium, which represent additional components of the placental barrier.

While the *ex vivo* dually perfused human placenta is most like the *in vivo* situation, these experiments are not without challenges, including the availability of fresh placentas, informed consent, and time-consuming procedures affected by a high leakage or failure rate. *In vitro* experiments with the BeWo cell line are easier to set up, can be carried out at times that are more convenient for lab personnel, and multiple results can be produced in less time, compared to the perfusions. Another disadvantage of the cell line is that in terms of monitoring interindividual differences, the BeWo cell line represents $n = 1$ individual. Pharmacogenetic differences in placental protein expression between individuals has been demonstrated; for example, significant variability in the amount of P-glycoprotein expressed in placental apical membranes from 12 term human placentas has been demonstrated (Nekhayeva et al., 2006). Placental perfusion experiments, on the other hand, will produce results more reflective of interindividual heterogeneity.

The placental transport using both the *in vitro* BeWo cell line and *ex vivo* placental perfusion was reported previously for selected allergens showing accumulation in the cells/placental tissue (Szepfalusi et al., 2000). In this work, however, the transport of four compounds (caffeine, benzoic acid, and glyphosate, plus the reference compound antipyrine) has been compared in both models of the placental barrier. With these data it has been possible to see that both models show excellent agreement in terms of ranking the compounds in order of their rate of transport, and both models serve as valuable tools necessary to predict the fetal exposure to potentially harmful substances that may affect fetal development.

In this work, it has been demonstrated that both of these models of placental transport (the BeWo cell line and *ex vivo* placental perfusions) show similar results in ranking the compounds according to their transfer rate. A step-wise approach should therefore be implemented for ideal planning of future research projects. The *in vitro* BeWo cell transport experiments should be employed as an initial screening tool, because these experiments are not as time consuming and still show high relevance to and predictability towards the data that would be collected by the *ex vivo* dually

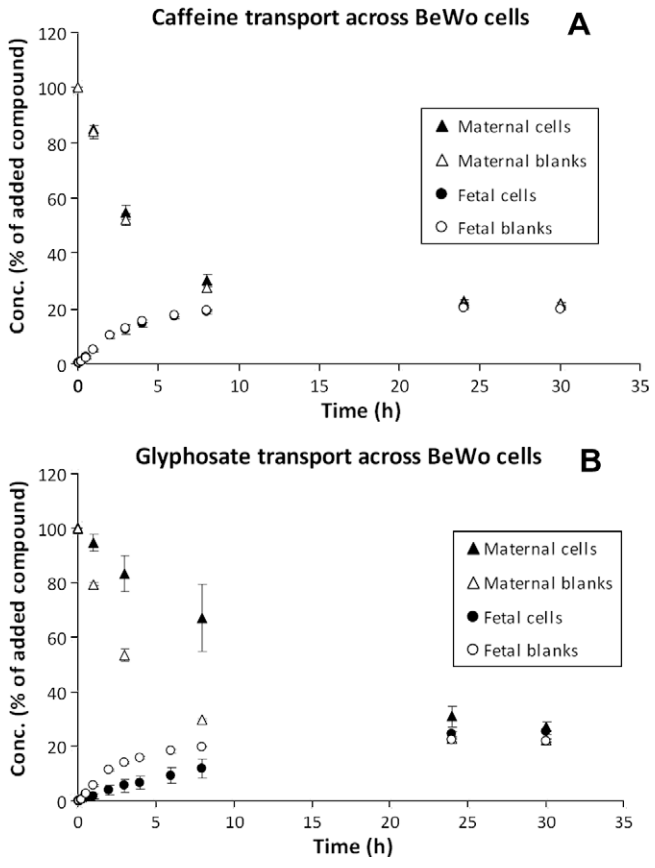

Fig. 4. Levels of caffeine (A) and glyphosate (B) in the apical (maternal) and basolateral (fetal) chambers during BeWo cell transport experiments (and blank Transwell inserts) over 24 h at 37 °C. The caffeine transport was studied in DMEM containing glutamine, penicillin/streptomycin, and 2% fetal bovine serum. 100 μL samples were drawn from the basolateral compartment as described in the text, but 10 μL samples were taken from the apical compartment.

perfused placenta experiments. If the results from BeWo cell experiments are consistent with expectations based on physical and chemical characteristics and comparison to analogous compounds, appropriate data classification from these *in vitro* experiments may be sufficient. Divergent results, the need for more complete information, or a desire to more closely approach the *in vivo* state would warrant further experimentation with the more sophisticated *ex vivo* dually perfused human placenta.

Acknowledgement

The authors acknowledge Lone Rosenquist for technical assistance and funding from ReProTect (LSHB-CT-2004-503257).

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