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Identification of P-Glycoprotein and Transport Mechanism of Paclitaxel in Syncytiotrophoblast Cells

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Abstract

When chemotherapy is administered during pregnancy, it is important to consider the fetus chemotherapy exposure, because it may lead to fetal consequences. Paclitaxel has become widely used in the metastatic and adjuvant settings for woman with cancer including breast and ovarian cancer. Therefore, we attempted to clarify the transport mechanisms of paclitaxel through blood-placenta barrier using rat conditionally immortalized syncytiotrophoblast cell lines (TR-TBTs). The uptake of paclitaxel was time- and temperature-dependent. Paclitaxel was eliminated about 50% from the cells within 30 min. The uptake of paclitaxel was saturable with K_m of 168 μM and 371 μM in TR-TBT 18d-1 and TR-TBT 18d-2, respectively. [^{3}H]Paclitaxel uptake was markedly inhibited by cyclosporine and verapamil, well-known substrates of P-glycoprotein (P-gp) transporter. However, several MRP substrates and organic anions had no effect on [^{3}H]paclitaxel uptake in TR-TBT cells. These results suggest that P-gp may be involved in paclitaxel transport at the placenta. TR-TBT cells expressed mRNA of P-gp. These findings are important for therapy of breast and ovarian cancer of pregnant women, and should be useful data in elucidating teratogenicity of paclitaxel during pregnancy.

Key Words: Paclitaxel, Pregnancy, Syncytiotrophoblast, TR-TBT cells, Blood-placental barrier, P-glycoprotein

INTRODUCTION

Cancer during pregnancy is uncommon, occurring in approximately 1/1,000 to 1/2,000 pregnancies (Van Calsteren *et al*., 2010). This incidence is expected to increase, given the trend for women to delay becoming first-time moms. Therefore, chemotherapy is regularly administered in pregnant women with cancer. When chemotherapy is administered during pregnancy, it is important to consider the fetus chemotherapy exposure, which may lead to fetal consequences including malformations, fetal growth retardation, and death. The placenta which regulates nutrient and waste exchange between the mother and the fetus may serve as a protective barrier for the fetus against maternal blood-borne toxins (Sai *et al*., 2008). Therefore, it is important to clarify the transfer of drugs from maternal blood to fetus across the blood-placenta barrier (BPB), which is composed of syncytiotrophoblast cells when chemotherapy is administered during pregnancy.

Among active drugs in breast, gynecological, and lung neoplasms that may occur during pregnancy, paclitaxel display a favorable toxicity profile during the second and third trimesters (Mir *et al*., 2010). To date, available human data suggest that the risk of congenital anomalies or premature birth as a result of paclitaxel use is low (De Santis *et al*., 2000; Sood *et al*., 2001; Méndez *et al*., 2003; Gonzalez-Angulo *et al*., 2004). Maternal side effects (mainly alopecia, nausea and vomiting, hematological toxicity) seem to be mild and manageable, as seen in non-pregnant women (Mir *et al*., 2010). These reports suggested the feasibility of use of pacalitaxel to pregnant cancer patients. Therefore, the aim of this study is to characterize certainly the uptake mechanism of paclitaxel at the blood-placenta barrier (BPB). Recently, TR-TBT cells are established from pregnant transgenic rats as an *in vitro* blood-placenta barrier (BPB) model (Kitano *et al*., 2004). It has been reported that the transport functions of the syncytiotrophoblast layer are similar in human and rat placenta (Takata and Hirano, 1997). Therefore, TR-TBTs are a good model for analysis of the placental transport of nutrients and chemicals. TR-TBTs exhibit typical properties of syncytiotrophoblast cells and TR-TBT 18d-1 and 18d-2 are derived from syncytiotrophoblast I and syncytiotrophoblast II, respectively (Kitano *et al*., 2004), so we adopted TR-TBT cells as a model cell lines for the present study.

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MATERIALS AND METHODS

Materials

Radiolabeled [o-benzamido-^{3}H]taxol ([^{3}H]paclitaxel, 1.0 mCi/ml) was obtained from Moravek Biochemicals (Mercury Lane, Brea, California, USA). Cyclosporine, verapamil, probenecid, cefmetazole, methotrexate and mitoxantrone were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals and reagents were commercial products of reagent grade.

Cell culture

TR-TBT cells were established from pregnant transgenic rat harboring the temperature-sensitive simian virus 40 large T-antigen genes at 18 days of gestation by Kitano *et al*. (2004). TR-TBT 18d-1 and 18d-2 have the histological characteristics of syncytiotrophoblast I and II, respectively (Kitano *et al*., 2004). The TR-TBT cells were cultured with Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS; Invitrogen), 100 U/ml penicillin (PC, Invitrogen), and 100 μg/ml streptomycin (SM; Invitrogen) at 33°C in a humidified atmosphere of 5% CO_2/air. On rat tail collagen type I-coated 24 well culture plates (IWAKI, Tokyo, Japan) initial seeding was done at 1×10^5 cells/ well and the cultures became confluent after seeding. Then the cells were incubated at 37°C for 2 days to deactivate SV40 temperature-sensitive T-antigen.

Functional studies

A confluent monolayer of TR-TBT cells was washed three times with 1 ml extracellular fluid (ECF) buffer consisting of 122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM K_2HPO_4, 10 mM D-glucose and 10 mM Hepes adjusted to pH 7.4 at 37°C. Uptake was initiated by applying 200 μl ECF buffer containing 1 μCi [^{3}H]paclitaxel at 37°C or 4°C in the presence or absence of inhibitors. After appropriate time periods, the applied solution was removed to terminate uptake and the cells were immersed in ice-cold ECF buffer. To measure the efflux of [^{3}H]paclitaxel in TR-TBTs, after incubating the cells with 1 μCi [^{3}H]paclitaxel at 37°C for 60 min, the reaction was stopped by adding ice-cold ECF buffer. Then the cells were incubated again with ECF buffer alone or containing unlabeled inhibitors at 37°C for designated time. After that, the cells were then solubilized in 750 μl of 1 N NaOH and radioactivity was measured in a liquid scintillation counter (LS6500; Beckman, Fullerton, CA, USA).

Data analysis

For kinetic studies, the Michaelis-Menten constant (K_m) and the maximum uptake rate (V_{max}) of [^{3}H]paclitaxel were estimated from Equation (1):

$$V=V_{max}\cdot C/(K_m+C)+K_d\cdot C \quad (1)$$

where V and C are the initial uptake rate of [^{3}H]paclitaxel at 5 min and the concentration of paclitaxel, V_{max} is the maximum uptake rate for the saturable component, and K_d is the first-order constant for the non-saturable component respectively.

Statistical analyses were carried out by one-way ANOVA with Dunnett's post-hoc test.

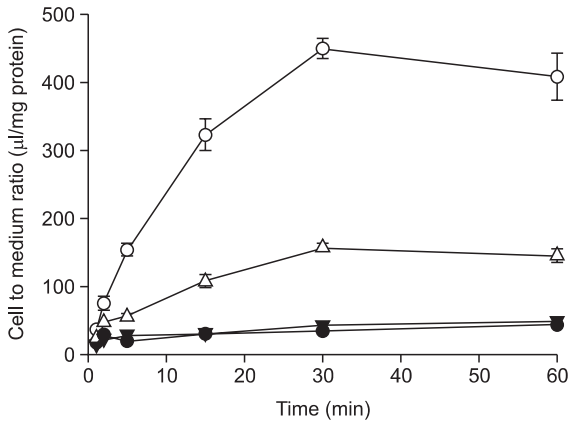

Fig. 1. Time course of [^{3}H]paclitaxel uptake by TR-TBT 18d-1 (circle) and 18d-2 (triangle) cells. [^{3}H]Paclitaxel uptake was performed at 37°C in ECF buffer (open) or 4°C (closed) and measured at designed time. Each point represents the mean ± SEM (n=3-4).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) analysis

Expression of rat mdr1a, rat mdr1b and human MDR1 by TR-TBT cells was analyzed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Total RNA from cultured TR-TBT cells and rat placenta was isolated by the RNeasy kit from Quiagen (Quiagen, Valencia, CA, USA) according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed by using oligo (dT) and Omniscript RT kit (Quiagen, Valencia, CA, USA). PCR for mdr1a, mdr1b, MDR1 was carried out using the following protocol. After an initial melting temperature of 95°C for 5 min and then amplification cycles of 10 sec of denaturation at 94°C, 30 sec of annealing at 55°C and 10 min of extension at 72°C were repeated for 40 cycles, followed by final extension. The sequences of sense and antisense primers were as follows: the sense primer was 5'-CAA CCA GCA TTC TTC ATA ATA-3' and antisense primer was 5'-CCC AAG GAT CAG GAA CAA TA-3' for mdr1a; the sense primer was 5'-CCT CCT TGG TCC TCT CAA-3' and antisense primer was 5'-TGT TTG GGG CTA AAT GTC-3' for mdr1b; the sense primer was 5'-GGC ATT TAC TTC AAA CTT GTC-3' and antisense primer was 5'-GCT TGG TGA GGA TCT CTC CAG CTT TG-3' for MDR1; the sense primer was 5'-TGA TGA CAT CAA GAA GGT GGT GAA G-3' and antisense primer was 5'-TCC TTG GAG GCC ATG TAG GCC AT-3' for GAPDH. The PCR products were subjected to electrophoresis on an agarose gel in the presence of ethidium bromide and visualized under ultraviolet light.

RESULTS

Time-course of [^{3}H]paclitaxel uptake and efflux by TR-TBT cells

We examined the uptake of [^{3}H]paclitaxel for 60 min in TR-TBTs in order to investigate characterization of paclitaxel transport at the BPB. [^{3}H]Paclitaxel uptake increased in a time-dependent manner it was linear for 5 min (Fig. 1). So, [^{3}H]paclitaxel uptake was evaluated at 5 min in the following kinetic and inhibition studies. [^{3}H]Paclitaxel uptake was markedly decreased at 4°C condition.

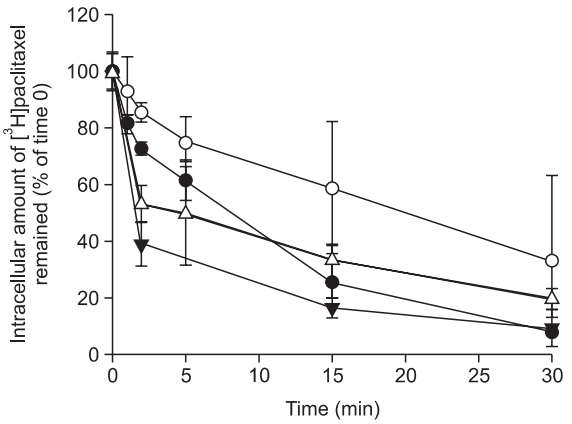

Fig. 2. Time course of [^{3}H]paclitaxel efflux by TR-TBT 18d-1 (circle) and 18d-2 (triangle) cells. [^{3}H]Paclitaxel efflux was performed with absence (open) or presence of 500 μM paclitaxel (closed). Each point represents the mean ± SEM (n=4).

In addition, [^{3}H]paclitaxel was eliminated by TR-TBTs. As shown Fig. 2, the amount of intracellular [^{3}H]paclitaxel was decreased in a time-dependent manner up to 30 min in the cells. [^{3}H]paclitaxel efflux was also significantly inhibited by the addition 500 μM unlabeled paclitaxel (Fig. 2). These results suggest that paclitaxel transport in TR-TBTs is involved in specific efflux transport systems.

Kinetic analysis of [^{3}H]paclitaxel uptake by TR-TBT cells

To characterize the kinetics of [^{3}H]paclitaxel uptake by TR-TBT cells, we analyzed the concentration dependence of [^{3}H]paclitaxel uptake. The transport process was saturable with Michaelis-Menten constant (K_m) of 168 μM and a maximum rate of uptake (V_{max}) of 216 pmol/mg protein/min in TR-TBT 18d-1 cells and K_m of 371 μM and V_{max} of 191 pmol/mg protein/min) in TR-TBT 18d-2 cells (Fig. 3).

Inhibitory effects of various compounds on [^{3}H]paclitaxel uptake by TR-TBT cells

To determine the substrate selectivity of paclitaxel transport system in TR-TBT cells, we performed inhibition studies with derivatives (Table 1). These experiments revealed [^{3}H]paclitaxel uptake was markedly inhibited by excess unlabeled paclitaxel and also by cyclosporine and verapamil, which are well-known substrate of P-glycoprotein (P-gp) transporter. [^{3}H]Paclitaxel uptake was not significantly inhibited by probenecid and cefmetazole, substrates of MRP4. Novobiocin, a substrate of organic anion transporter (OAT) did not significantly inhibited the uptake of [^{3}H]paclitaxel. And several amino acids, adenosine and glycine did not inhibited [^{3}H]paclitaxel uptake.

The gene expression of p-glycoprotein in TR-TBT cells, rat and human placenta

Total RNA from cultured TR-TBTs and rat placenta was isolated by the RNeasy kit from Quiagen, according to the manufacturer's instruction. The expression of rmdr1a, rmdr1b and hMDR1 was analyzed by RT-PCR using total RNA isolated from rat placenta and TR-TBTs (Fig. 4). The rmdr1a at 97 bp, rmdr1b at 846 bp and hMDR1 at 502 bp were amplified in rat placenta as a positive control.

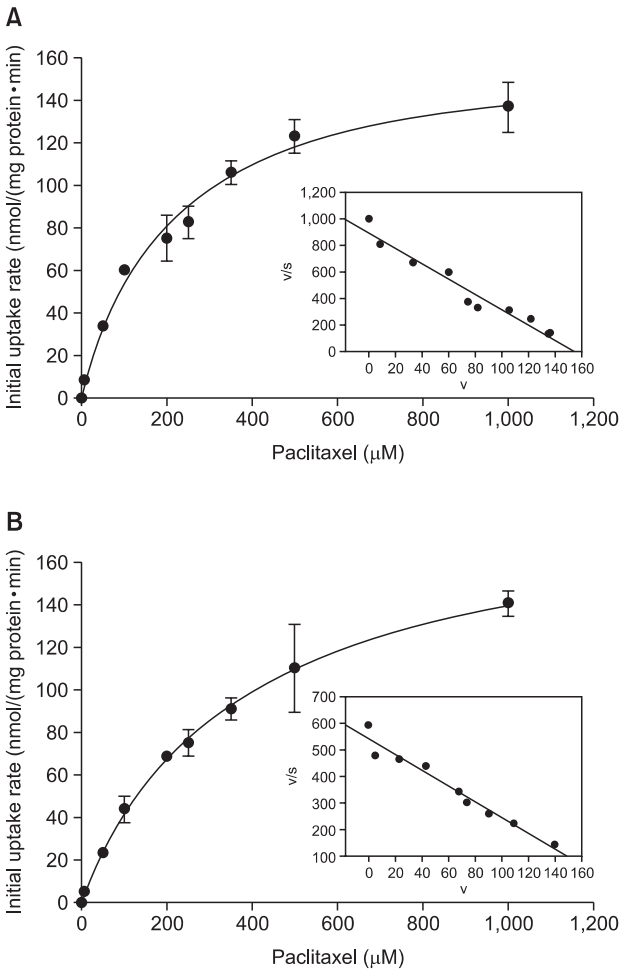

Fig. 3. Uptake of [^{3}H]paclitaxel was measured in TR-TBT 18d-1 (A) and 18d-2 (B) cells with 5 min incubation in the presence of 0-500 μM unlabeled paclitaxel at pH 7.4 and 37°C. The data (insert) are shown as an Eadie-Hofstee plot of the saturable component. The data represent the mean ± SEM (n=3-4).

DISCUSSION

The goal of this study is to investigate transport mechanisms of paclitaxel from maternal uptake across to the fetus through the blood placenta barrier. We used TR-TBT 18d-1 and 18d-2 as an *in vitro* model of the BPB.

Conditionally immortalized rat syncytiotrophoblast cell line, TR-TBT was established from pregnant transgenic rats harboring the temperature-sensitive simian virus 40 large T-antigen genes at 18 days of gestation by Faculty of Pharmacy, Keio University (Kitano *et al*., 2002). The cells exhibited the typical characteristics of *in vivo* syncytiotrophoblast (Kitano *et al*., 2004). Especially, they were testified to form a polarized layer of cells with the apical and basolateral membranes having functional similarities to those in rat placenta (Kitano *et al*., 2002; Kitano *et al*., 2004). In addition, TR-TBT cells express many transporter genes found in human placenta and also exhibit the uptake activity of taurine and GABA through the several transporters. Recently, our group characterized the BPB uptake mechanism of choline and 6-mercaptopurine (6-MP)

Table 1. Effect of several transporter inhibitors on [^{3}H]paclitaxel uptake in TR-TBT cells

Substrate	Concentration (mM)	[^{3}H]Paclitaxel uptake (%)	
		TR-TBT 18d-1	TR-TBT 18d-2
Control		100.0 ± 6.0	100.0 ± 4.0
Paclitaxel	0.5	23.8 ± 1.1***	13.8 ± 1.8***
Cyclosporin	0.5	20.3 ± 1.0***	54.4 ± 2.7***
Verapamil	1	42.1 ± 1.8***	22.7 ± 3.9***
Probenecid	1	106.0 ± 8.0	108.0 ± 6.0
Cefmetazole	1	110.0 ± 6.0	101.0 ± 10.0
Methotrexate	1	75.2 ± 10.4*	88.0 ± 10.3
Mitoxantrone	1	91.0 ± 6.9	89.6 ± 9.5
Novobiocin	1	93.6 ± 8.5	102.0 ± 13.0
Adenosine	1	92.9 ± 4.0	105.0 ± 6.0
Glycine	1	90.7 ± 5.2	85.3 ± 8.5

[^{3}H]Paclitaxel uptake by TR-TBT cells was measured in the absence (control) or presence of compounds for 5 min at 37°C. Each value represents the mean ± S.E.M. (n=4). *p<0.05, **p<0.01, ***p<0.001; significantly different from control.

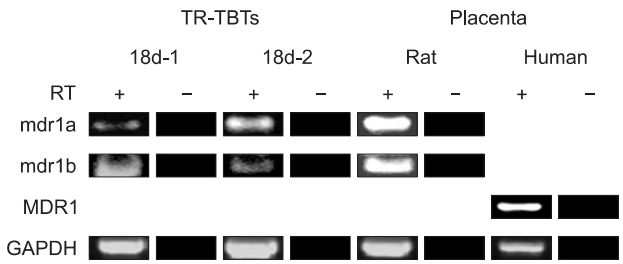

Fig. 4. Expression of mdr1a, mdr1b, MDR1 and GAPDH by RT-PCR in TR-TBT cells and placenta in rat and human. Total RNA (1 μg) was reverse-transcribed and cDNA (0.1 μg) was amplified by PCR. Products were electrophoresed on 5% acrylamide gel and visualized by ethidium bromide staining. Products of isolated cells were observed at the expected sizes. (+) and (-) represent the presence or absence of reverse transcriptase (RT), respectively.

via choline transporter-like protein 1 (CTL1) and equilibrative nucleoside transporters (ENTs) using these cells, respectively (Lee *et al*., 2009; Lee *et al*., 2011). Also, it is reported that uridine and adenosine are taken up via ENTs in TR-TBT cells (Chishu *et al*., 2008; Sato *et al*., 2009). Therefore, TR-TBT cells were considered to be a suitable model for the analysis of paclitaxel transport activities in the BPB. In rat, the labyrinthine part of the placenta is the principal site of maternal-fetal exchange. The labyrinthine wall is composed of two syncytiotrophoblast layers, the maternal-side (SynI) and fetal-side syncytiotrophoblast layers (SynII) (Kitano *et al*., 2002). TR-TBT 18d-1 and 18d-2 have the histological characteristics of syncytiotrophoblast I and II respectively (Kitano *et al*., 2004). According to these reason, we investigated paclitaxel transport using both cell lines in this study.

It has been reported that paclitaxel is a substrate of P-gp (Murray *et al*., 2012). Indeed, the P-gp plays a critical role in protecting the organism against xenobiotics, decreasing their tissue accumulation in an ATP-dependent manner (Fromm, 2004). In our results, mRNA of P-gp, mdr1a and mdr1b was expressed in TR-TBT cells and rat placenta (positive control) (Fig. 4). [^{3}H]Paclitaxel uptake was time dependency and inhibited unlableled paclitaxel (Fig. 1). Therefore, it is suggested that paclitaxel can be delivered by specific transporter from TR-TBT cell lines. Also, about 50% of the amount of intracellular [^{3}H]paclitaxel was decreased in a time dependent manner up to 30 min in the TR-TBT cells (Fig. 2). [^{3}H]Paclitaxel efflux was also significantly inhibited by the addition 500 μM unlabeled paclitaxel (Fig. 2). Verapamil and cyclosporine are well-known inhibitors of P-gp transport system(s) (Staud *et al*., 2012), respectively, they had significant inhibitory effect, indicating that p-gp is involved in paclitaxel uptake by TR-TBT cells. Breast cancer resistance protein (BCRP) transports a variety of drugs including mitoxantrone and cefmetazole (Mao, 2008), however, these drugs had no effect on paclitaxel transport in TR-TBT cells. [^{3}H]Paclitaxel uptake was not significantly inhibited by probenecid and cefmetazole, substrates of MRP4. Novobiocin, a substrate of organic anion transporter (OAT) did not significantly inhibited the uptake of [^{3}H]paclitaxel. And several amino acids, adenosine and glycine did not inhibited [^{3}H]paclitaxel uptake (Table 1). Kinetic analysis revealed that paclitaxel uptake by TR-TBT cells was saturable (Fig. 3). The K_m value for paclitaxel uptake in TR-TBT 18d-1 and 18d-2 were approximately 168 μM and 371 μM, respectively (Fig. 3). Taken together, P-gp might play an important role in paclitaxel transport across the BPB from maternal plasma. Indeed, it has been reported that intravenous administration of paclitaxel to pregnant dams revealed a 16-fold higher trans-placental transfer rate in *mdr1a/1b* (-/-) knockout mice than in wild-type mice (Smit *et al*., 1999). In a recent report, paclitaxel could not be detected in fetal plasma at ninety minutes after IV injection in pregnant mice (Van Calsteren *et al*., 2011). Therefore, we hypothesize that the placental P-gp reduces the trans-placental transfer of paclitaxel, making their clinical use possible during the 2nd and 3rd trimesters of pregnancy. So far, 25 cases have been described using paclitaxel during pregnancy (Mir *et al*., 2010) and no malformation was reported. Also, data concerning maternal hematologic toxicity were not available in most cases (Mir *et al*., 2010). TR-TBT cells were originated from rat. It has been reported that transport functions in the BPB are similar in human and rat placenta (Takata and Hirano, 1997). In our results, mRNA of P-gp, was expressed in TR-TBT cells, rat and human placenta (Fig. 4). Therefore, we expected to exist similar mechanisms of pacli-

taxel transport in human placenta.

In conclusion, P-gp expressed at the BPB is involved in the transport of paclitaxel. Our findings are important for therapy of breast and ovarian cancer of pregnant women, and should be useful data in elucidating teratogenicity of paclitaxel during pregnancy.

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REFERENCES

Chishu, T., Sai, Y., Nishimura, T., Sato, K., Kose, N., Nakashima, E. (2008) Potential of various drugs to inhibit nucleoside uptake in rat syncytiotrophoblast cell line, TR-TBT 18d-1. *Placenta* **29**, 461-467.

De Santis, M., Lucchese, A., De Carolis, S., Ferrazani, S., Caruso, A. (2000) Metastatic breast cancer in pregnancy: first case of chemotherapy with docetaxel. *Eur. J. Cancer Care* **9**, 235-237.

Fromm, M. F. (2004) Importance of P-glycoprotein at blood-tissue barriers. *Trends Pharmacol. Sci.* **25**, 423-429.

Gonzalez-Angulo, A. M., Walters, R. S., Carpenter, R. J. Jr, Ross, M. I., Perkins, G. H., Gwyn, K., Theriault, R. L. (2004) Paclitaxel chemotherapy in a pregnant patient with bilateral breast cancer. *Clin. Breast Cancer* **5**, 317-319.

Kitano, T., Iizasa, H., Hwang, I. W., Hirose, Y., Morita, T., Maeda, T., Nakashima, E. (2004) Conditionally immortalized syncytiotrophoblast cell lines as new tools for study of the blood-placenta barrier. *Biol. Pharm. Bull.* **27**, 753-759.

Kitano, T., Iizasa, H., Terasaki, T., Asashima, T., Matsunaga, N., Utoguchi, N., Watanabe, Y., Obinata, M., Ueda, M., Nakashima, E. (2002) Polarized glucose transporters and mRNA expression properties in newly developed rat syncytiotrophoblast cell lines, TR-TBTs. *J. Cell. Physiol.* **193**, 208-218.

Lee, N. Y., Choi, H. M., Kang, Y. S. (2009) Choline transport via choline transporter-like protein 1 in conditionally immortalized rat syncytiotrophoblast cell lines TR-TBT. *Placenta* **30**, 368-374.

Lee, N. Y., Sai, Y., Nakashima, E., Ohtsuki, S., Kang, Y. S. (2011) 6-Mercaptopurine transport by equilibrative nucleoside transporters in conditionally immortalized rat syncytiotrophoblast cell lines TR-TBTs. *J. Pharm. Sci.* **100**, 3773-3782.

Mao, Q. (2008) BCRP/ABCG2 in the placenta: expression, function and regulation. *Pharm. Res.* **25**, 1244-1255.

Méndez, L. E., Mueller, A., Salom, E., González-Quintero, V. H. (2003) Paclitaxel and carboplatin chemotherapy administered during pregnancy for advanced epithelial ovarian cancer. *Obstet. Gynecol.* **102**, 1200-1202.

Mir, O., Berveiller, P., Goffinet, F., Treluyer, J. M., Serreau, R., Goldwasser, F., Rouzier, R. (2010) Taxanes for breast cancer during pregnancy: a systematic review. *Ann. Oncol.* **21**, 425-426.

Murray, S., Briasoulis, E., Linardou, H., Bafaloukos, D., Papadimitriou, C. (2012) Taxane resistance in breast cancer: mechanisms, predictive biomarkers and circumvention strategies. *Cancer Treat. Rev.* **38**, 890-903.

Sai, Y., Nishimura, T., Shimpo, S., Chishu, T., Sato, K., Kose, N., Terasaki, T., Mukai, C., Kitagaki, S., Miyakoshi, N., Kang, Y. S., Nakashima, E. (2008) Characterization of the mechanism of zidovudine uptake by rat conditionally immortalized syncytiotrophoblast cell line TR-TBT. *Pharm. Res.* **25**, 1647-1653.

Sato, K., Sai, Y., Nishimura, T., Chishu, T., Shimpo, S., Kose, N., Nakashima, E. (2009) Influx mechanism of 2',3'-dideoxyinosine and uridine at the blood-placenta barrier. *Placenta* **30**, 263-269.

Smit, J. W., Huisman, M. T., van Tellingen, O., Wiltshire, H. R., Schinkel, A. H. (1999) Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. *J. Clin. Invest.* **104**, 1441-1447.

Sood, A. K., Shahin, M. S., Sorosky, J. I. (2001) Paclitaxel and platinum chemotherapy for ovarian carcinoma during pregnancy. *Gynecol. Oncol.* **83**, 599-600.

Staud, F., Cerveny, L., Ceckova, M. (2012) Pharmacotherapy in pregnancy; effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. *J. Drug Target.* **20**, 736-763.

Takata, K., Hirano, H. (1997) Mechanism of glucose transport across the human and rat placental barrier: a review. *Microsc. Res. Tech.* **38**, 145-152.

Van Calsteren, K., Heyns, L., De Smet, F., Van Eycken, L., Gziri, M. M., Van Gemert, W., Halaska, M., Vergote, I., Ottevanger, N., Amant, F. (2010) Cancer during pregnancy: an analysis of 215 patients emphasizing the obstetrical and the neonatal outcomes. *J. Clin. Oncol.* **28**, 683-689.

Van Calsteren, K., Verbesselt, R., Van Bree, R., Heyns, L., de Bruijn, E., de Hoon, J., Amant, F. (2011) Substantial variation in transplacental transfer of chemotherapeutic agents in a mouse model. *Reprod. Sci.* **18**, 57-63.