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Abstract: The objective of this study was to investigate the functional expression of the efflux transporter, P-glycoprotein (P-gp), in primary cultures of human cytotrophoblasts and BeWo cell monolayers. Uptake studies with primary cultures of human cytotrophoblasts or BeWo cells were conducted with calcein-AM and vinblastine (P-gp markers) or fluorescein (MRP marker) in the presence of specific P-gp or MRP inhibitors. Results showed that the accumulation of P-gp substrates calcein-AM and vinblastine by BeWo cells or primary cultures of human cytotrophoblasts was significantly enhanced in the presence of a typical P-gp inhibitor, cyclosporin-A, or other inhibitors such as quinidine, verapamil and dipyridamole. MRP inhibitors had no effect on the accumulation of calcein or fluorescein by BeWo cells. Western blots confirmed the presence of multidrug resistant gene product 1 (MDR1) in both, primary cultures of human cytotrophoblasts and BeWo cells. This study demonstrates functional P-gp in term human trophoblasts and further supports the use of primary cultures of human cytotrophoblasts and BeWo cells as in vitro models of the trophoblast to investigate mechanisms regulating drug distribution across the placenta.

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Functional Expression of P-Glycoprotein in Primary Cultures of Human Cytotrophoblasts and BeWo Cells

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Introduction

The syncytiotrophoblast layer comprises the human placental barrier and plays an important role in controlling the passage of blood-borne substances from mother to fetus [1]. The primary mechanism of transport of substances from the mother to fetus is largely considered to be via passive diffusion, although specific carrier-mediated processes have been identified [2,3]. In addition, the transcellular mechanism of drug transport across the placenta is possible for drugs having a lipophilic or hydrophobic nature[1-3].

P-glycoprotein, a 170-kDa transmembrane glycoprotein (multidrug resistant gene product 1 or MDR1), and multidrug resistant associated protein (MRP), a 190-kDa protein, have been established to be active efflux mechanisms in cells from multidrug-resistant cell lines [4]. P-gp can confer multidrug resistance by actively extruding a wide range of structurally unrelated, amphiphilic or hydrophobic drugs from the cell. Most of the substrates for P-gp are toxic compounds of natural or semisynthetic origin [5]. P-gp is expressed in various normal human tissues, such as small intestine, kidney, liver, and capillary endothelial cells of brain and testes [6-8]. Based on the tissue distribution, P-gp has been proposed to have a significant role in the protection of organisms against toxic xenobitics [9,10]. MRP is now known as a family of at least six distinct proteins also with polyspecific substrate efflux properties and some overlapping substrate specificity with P-gp [11,12].

Immunohistochemical studies have shown that P-gp is also expressed in the placenta [6]. Nakamura et al. [13] using immunoblotting experiments also showed the expression of P-gp specifically in the human cyto- and syncytio-trophoblasts. Although P-gp in the analogous transporting trophoblast layer (labyrinth) is speculated to play a signficant role in the protection of the rodent fetus from toxic compounds [14], its function, such as transporting activity and substrate specificity in the human cyto- and syncytio-trophoblasts, remain unknown. Also, currently no information exists regarding the presence of a functional multidrug resistant associated protein (MRP) in the placenta.

The objectives of this study were to determine if P-gp and MRP expressed by human trophoblasts is functional by employing the in vitro systems, primary cultures of human cytotrophoblasts which spontaneously aggregate to form a syncytiotrophoblast, and the trophoblast-like BeWo cell line, which is comprised of a mix of predominantly cytotrophoblasts and a few syncytiotrophoblasts. Our previous studies have described the use of both human cytotrophoblasts and BeWo cell monolayers to investigate normal human trophoblast transport and metabolism functions [3,16-19]. Demonstration of the efflux mechanism in these in vitro systems provides potential tools to investigate the roles and contributions trophoblast transporters in drug distribution across the human placenta.

Materials and Methods

Chemicals

Calcein-AM and fluorescein were obtained from Sigma Chemicals (St Louis, MO). ^{3}H-vinblastine was obtained from Moravek Biochemicals (Brea, CA). All other chemicals and reagents were obtained from Sigma or Fisher and used without further purification.

BeWo cell culture:

The BeWo cell line was originally derived from a human choriocarcinoma [20]. The BeWo clone (b30) was obtained from Dr. Alan Schwartz (Washington University, St. Louis, MO). The cell culture protocol previously described was followed [15]. Briefly, the cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 0.37% sodium bicarbonate and 1% antibiotics (10000 U/ml penicillin and 10 mg/ml streptomycin). The cells were maintained in 175-cm^2 flasks at pH 7.4 under 5% CO_2 and 95% humidity at 37°C. The cells were harvested by exposure to a trypsin-EDTA solution (0.25% trypsin and 0.02% EDTA in Hanks' balanced salt solution) and passed onto 12-well tissue culture plates. With the seeding density of 10,000 cells/cm^2, the cells formed monolayers between 3 and 4 days and were used for experiments at that time.

Primary Cultures of human cytotrophoblasts:

The study protocol and use of human placentas in this study was approved by The University of Kansas Advisory Committee on Human Experimentation (ACHE #8904). Term placentas were obtained from unremarkable pregnancies of nonsmoking volunteers. The procedure for isolation and primary culture of human cytotrophoblasts was as previously detailed [17].

Functional expression of P-gp:

Experiments were conducted to determine the effect of cyclosporin-A (CSA) concentration on the uptake of calcein-AM by BeWo cells. The BeWo cells at confluence were washed three times with the assay buffer of the following composition (mM): NaCl (122), $NaHCO_3$ (25), KCl (3), $MgSO_4$ (1.2), KH_2PO_4 (0.4), $CaCl_2$ (1.4), D-glucose (10), Hepes (10). The cells were incubated with 0.5 ml of calcein-AM (1.0 µM) in the presence of CSA (0, 0.3, 1.0, 3.0 and 10 µM) for 30 minutes at 37°C. Following incubation, the test buffer was aspirated away and the cells washed three times with ice cold buffer. The cells were lysed overnight with 0.5 ml of a 0.2 N NaOH solution that included 0.2% triton X-100. Cellular uptake of calcein-AM was determined by fluorescence spectroscopy (SLM Instruments Inc. Urbana, IL) at excitation and emission wavelengths of 488 and 530 nm, respectively. The samples were normalized for protein content using a BCA Protein Assay Reagent Kit (Pierce) with bovine serum albumin as the reference standard.

In a separate series of experiments, cells were incubated with 0.5 ml of calcein/AM (1.0 µM) in the presence of either a single P-gp inhibitor (10 µM CSA or 100 µM of quinidine or verapamil or dipyridamole) or a single MRP inhibitor (10 µM indomethacin or 100 µM of genistein or valproic or probenecid) for 30 minutes at 37°C. The cells were then harvested as described above and assayed for accumulation of the fluorescent calcein.

Experiments were carried out to determine the effect of P-gp inhibitors on the accumulation of ^{3}H-vinblastine, an alternative P-gp substrate, in BeWo cells using the protocol for Calcein-AM described above. Uptake of ^{3}H-vinblastine (47.2 nM) by BeWo cells was studied in the presence of 10 µM CSA or 100 µM of quinidine or verapamil or dipyridamole. Cellular uptake of ^{3}H-vinblastine was measured by scintillation counting (Beckman Instruments) and was normalized for protein content.

Uptake studies with primary cultures of human cytotrophoblasts were conducted in an identical fashion. Briefly, the isolated trophoblast cells were grown in 12-well plates in Dulbecco's modified Eagle's medium containing 20% fetal bovine serum at a seeding density of 50,000 cells/cm^{2}. Experiments were initiated 4-5 days post-seeding. The accumulation of 0.5 ml of calcein-AM (1.0 µM) or ^{3}H-vinblastine (47 nM) was investigated in the presence of 10 µM CSA or 100 µM dipyridamole. Cellular uptake of calcein-AM or ^{3}H-vinblastine was determined by methods described above.

Functional expression of MRP:

Fluorescein is a substrate for MRP [21] and was therefore used in these studies. Cellular uptake of fluorescein by BeWo cells, was determined in the presence of MRP inhibitors such as probenecid (100 µM), indomethacin (100 µM), genestein (10 µM) and valproic acid (100 µM). Following a 30 minutes pre-incubation with the inhibitors, the cells were incubated with 0.5 ml of fluorescein (100 µM) in the presence or absence (control group) of the inhibitors. The experimental procedure was similar to the one described above. Cellular uptake of fluorescein was determined by fluorescence spectroscopy at excitation and emission wavelengths of 488 and 530 nm, respectively, and normalized for protein content.

Immunochemical characterization:

Membrane preparations (0.5 to 10-µg placental microsome protein or 0.05-µg BeWo or primary trophoblast cells) were subjected to SDS-PAGE, 12% acrylamide Tris-glycine. All membrane samples were heated to 100°C for 10 min and centrifuged at 10,000 g for 5 min. Molecular weight markers (250 kDa to 4 kDa) and purified bovine serum albumin (0.5 mg) served as controls. Electrophoresis of microsomes with SDS-PAGE was monitored at 95 volts for 2.5 hr. Following electrophoresis, protein was either transferred to PVDF or polyacrylamide gels and exposed to Coumassie blue stain (0.1% Coumassie blue, 45% methanol, 2% acetic acid) for one hour. Following SDS-PAGE, membrane proteins were transferred electrophoretically to PVDF membranes (45 volts, 2 hr). After electrophoresis transfer, PVDF membranes were immersed in Ponceau S staining solution for 5 min to verify the presence of membrane protein. The Ponceau stain was removed in an aqueous solution containing 10% acetic acid (v/v) for approximately 4-5 min. To block non-specific binding of the monoclonal antibody (C219; Signet, Deadham, MA), membranes were incubated for two hours with phosphate buffered saline, pH 7.4 containing 10% dry milk (w/v), 2% bovine serum albumin (w/v) and 0.1% tween-20. The membranes were then incubated with monoclonal antibody to P-

gp (10 mg/ml) or a pre-immune mouse antibody (non-specific IgG, 10 mg/ml) in 10 mM Tris-HCl, pH 7.4, containing 0.1% tween-20. All membrane protein bound to PVDF membranes was incubated overnight at room temperature with the polyclonal antibody or the nonspecific IgG. A secondary antibody IgG peroxidase conjugate specific for the P-gp monoclonal antibody, was diluted 1:80,000 in 10 mM Tris-HCl containing 0.05% tween-20 and incubated with the PVDF membrane for one hour at 25°C. P-gp was detected by enhanced chemiluminescence by incubating PVDF immunoblots at 25°C for 45 sec and then exposed to Hyperfilm-ECl.

Data Analysis

Data are expressed as means ± SD for at least four replicates. The comparison between mean values within each experimental series was performed by one-way analysis of variance by using Dunnett's test to compare several treatments against an untreated control. The 0.05 level of probability was used as the criterion of significance.

Results

The effect of different concentrations of CSA on the cellular uptake of calcein-AM is shown in Figure 1. Uptake of calcein-AM, measure by calcein accumulation, increased significantly ($p<0.05$) and in a concentration-dependent manner with increasing concentrations of CSA. A 10 µM concentration of CSA resulted in the highest uptake of calcein-AM by BeWo cells. The functional presence of P-gp in BeWo cells was further affirmed by studying the uptake of an alternative substrate, ^{3}H-vinblastine, in the presence of selected P-gp inhibitors as shown in Figure 2. The uptake of ^{3}H-vinblastine was significantly higher in the presence of P-gp inhibitors such as quinidine, verapamil and dipyridamole indicating the presence of P-gp activity in BeWo cells.

The uptake of calcein-AM and ^{3}H-vinblastine by primary cultures of human cytotrophoblasts was investigated in the presence of two P-gp inhibitors CSA and dipyridamole as shown in Figures 3A and 3B, respectively. The uptake of both substrates was significantly enhanced ($p<0.05$) in the presence of the P-gp inhibitors indicating functional activity of P-gp in primary cultures of human cytotrophoblasts, consistent with the observations with BeWo cells.

Both the primary cultures of human cytotrophoblasts and BeWo cells were shown to retain expression of MDR1. The immunoblot shown in Figure 4 was generated with the anti-human C219 monoclonal antibody and confirmed typical bands for protein corresponding to P-gp from the BeWo cell line and from four different preparations of primary cultures of cytotrophoblasts.

The functional expression of MRP in BeWo cells was investigated by studying the uptake of the MRP substrate, fluorescein, by BeWo cells in the presence of MRP inhibitors, genestein, valproic acid, probenecid and indomethacin. As illustrated in Figure 5, the cellular uptake of fluorescein was not significantly enhanced in the presence of MRP inhibitors, indicating the absence of MRP in BeWo cells. Further, data summarized in Table 1 confirmed that a selection of P-gp inhibitors, but not MRP inhibitors, effectively stimulated calcein accumulation ruling out an apparent functional MRP activity. Therefore, these results verified

that the measured increase in fluorescence in our experiments with calcein/AM was due to impairment of P-gp-mediated efflux of calcein/AM rather than MRP-mediated efflux of the fluorescent calcein.

Discussion

P-glycoprotein or MDR1 is an efflux membrane pump with broad substrate specificity for a variety of structurally diverse chemicals. Drugs such as the vinca alkaloids, anthracyclines, taxanes and cyclosporin A have been shown to be P-gp inhibitors [22]. The transporting P-gps are generally overexpressed in tumor cells, conferring resistance against cytotoxic agents and are associated with specialized normal tissue barriers (e.g., blood-brain barrier, blood-testis barrier, blood-ovarian barrier, placenta, gastrointestinal epithelium, etc.) [6-8].

Although the placenta has been considered to be an inefficient barrier to drug distribution between maternal and fetal circulations [23], Lankas et al. [14] recently provided evidence of the importance of placental P-gp in a spontaneous "knockout" mice. Their study suggested that the placenta may play more than a passive role in regulating chemical distribution and consequently, differences in blood levels of chemicals between the mother and fetus to the point of influencing fetal development. Specifically, 100% of the fetuses from placental P-gp knockouts exhibited birth defects on chemical exposure at critical times during pregancy. Fetuses from mice with normal placental P-gp expression were exposed to a substantially lower chemical burden and completely protected from birth defects. Mylona et al. [24] have reported abundant expression of the MDR1 gene in the human placenta, particularly in the cytotrophoblasts throughout the course of pregnancy. An alternative mechanism, a MRP, also purportedly is expressed in the human placenta [25]. Therefore, the expression of the MDR1 gene and MRP may also provide the human placenta with potential mechanisms to regulate fetal exposure to drugs and chemicals.

We report here, specific uptake studies conducted to confirm the functional activity of P-gp in the human trophoblast using human cell culture systems. Calcein-AM was chosen as one of the markers to determine P-gp activity in BeWo cells. In the absence of P-pg influences, calcein-AM is a non-fluorescent, highly lipid soluble dye that can rapidly permeate the plasma membrane of normal cells. Once inside the cells, the ester bonds are cleaved by the endogenous esterases converting calcein-AM to fluorescent calcein [26]. The electronegative calcein generated in the cytoplasm is a substrate for MRP, not Pgp, and is retained in the cell in the absence of MRP [27]. The presence of CSA, a known P-gp inhibitor [28] increased the accumulation of calcein in BeWo cells in a concentration-dependent manner indicating significant P-gp activity. Accumulation of the alternative P-gp substrate, vinblastine was also enhanced in the presence of other known P-gp inhibitors.

The synctiotrophoblast surrounding the chorionic villi forms the primary barrier between the mother and fetus. The functional expression of P-gp in the trophoblast cells was investigated using human cytotrobhoblasts, which fully aggregate to form a syncytiotrophoblast in primary culture. As seen with BeWo cells, which is a mixture of primarily cytotrophoblasts with a few synctiotrophoblasts [15], the uptake of P-gp substrates vinblastine and calcein-AM by the primary cultures, was also significantly enhanced in the presence of other P-gp inhibitors. The

biochemical evidence for the presence of P-gp in BeWo cells and the primary cultures was confirmed by immunoblotting experiments. The C219 monoclonal antibody used in these experiments is specific for MDR1 [29,30] and the positive findings were in good agreement with the literature [24,31] for normal human trophoblasts.

The presence of MRP-like activity in BeWo cells was investigated using fluorescein as a substrate. Fluorescein has been used previously as a marker for similar types studies [21]. However, the presence of MRP inhibitors such as valproic acid, genestein, probenecid and indomethacin, did not result in significant increase in fluorescein accumulation. As described above, calcein-AM is a substrate for P-gp and calcein a substrate for MRP [27]. In our studies, only the P-gp inhibitors, and not the MRP inhibitors, stimulated accumulation of calcein and further confirmed the fluorescein study results. Our studies were consistent with the findings of Sugawara et al. [32] who have shown that MRP is not expressed in the placenta and in contrast to Flens et al [25] who suggested the presence of MRP in human placenta. We have not yet performed immunoblots for MRP which may show expression of a protein that was not active in our study. The possible presence of multiple isoforms of MRP [11,12] in the trophoblasts creates a more complex investigation and will have to be considered in future studies.

In summary, this study reports the functional expression of P-gp in the BeWo cell monolayers and primary cultures of human cytotrophoblasts, in vitro systems representing the human trophoblast. The results further emphasize some of the biochemical similarities of the BeWo cells and the primary cultures of normal human cytotrophoblasts and affirm the use of the cell line for some investigations of trans-trophoblast transport of nutrients and drugs. Recent reports suggest that endogenous steroids including the hormone of pregnancy, progesterone, may regulate P-gp function [33]. Therefore, our results will form the basis for further investigation of the role of efflux systems in regulating the distribution of endogenous steroids and xenobiotics across the placenta, and their possible role in regulating P-gp function in the human trophoblast.

References

1. Stulc J. Extracellular transport pathways in the haemochorial placenta. Placenta 1989; 10: 113-119.

2. Ganapathy V, Prasad P, Ganapathy M, Leibach F. Drugs of abuse and placental transport. Adv.Drug.Del.Rev. 1999; 38: 99-110.

3. Knipp G, Audus KL, Soares, MJ. Nutrient transport across the placenta. Adv.Drug.Del.Rev. 1999; 38: 17-40.

4. Higgins CF. ABC transporters: from microorganisms to man. Annu.Rev.Cell Biol. 1992; 8: 67-113.

5. Chin KV, Pastan I, Gottesman MM. Function and regulation of the human multidrug resistance gene. Adv.Cancer Res. 1993; 60: 157-180.

6. Sugawara I, Kataoka I, Morishita Y, Hamada H, Tsuruo T, Itoyama S, Mori S. Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK 16. Cancer Res. 1988; 48: 1926-1929.

7. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc.Natl.Acad.Sci.U.S.A. 1987; 84: 7735-7738.

8. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: evidence for localization in brain capillaries and cross reactivity of one antibody with a muscle protein. J.Histochem.Cytochem. 1989; 37: 159-164.

9. Gottesman MM, Pastan I, Ambudkar SV. P-glycoprotein and multidrug resistance. Curr. Opin. Genet. Develop. 1996; 6:610-617.

10. Schinkel AH, Wagenaar E, Mol CAAM, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J. Clin. Invest. 1996; 97:2517-2524.

11. Kool, M., de Haas, M., Scheffer, G.L., Scheper, R.J., van Eijk, M.J., Juijn, J.A., Baas, F., and Borst, P. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res. 1997; 57:3537-3547.

12. Kool, M., van der Linden, P.A., de Haas, M., Baas, F., and Borst, P. Expression of human MRP6, a homologue of the multidrug resistance protein gene MRP1, in tissues and cancer cells. Cancer Res. 1999; 59:175-82.

13. Nakamura Y, Ikeda S, Furukawa T, Sumizawa T, Tani A, Akiyama S, Nagata Y. Function of P-glycoprotein expressed in placenta and mole. Biochem.Biophys.Res.Commun. 1997; 235: 849-853.

14. Lankas GR, Wise LD, Cartwright ME, Pippert T, Umbenhauer DR. Placental P-glycoprotein deficiency enhances susceptibility to chemically induced birth defects in mice. Reprod.Toxicol. 1998; 12: 457-463.

15. Liu F, Soares MJ, Audus KL. Permeability properties of monolayers of the human trophoblast cell line BeWo. Am.J.Physiol. 1997; 273: C1596-C1604.

16. Chandorkar GA, Audus KL. Transport of opioid peptides across BeWo cell monolayers, a model of the placental barrier. Pharm.Sci. 1998; 1: S-386.

17. Kenagy J, Liu F, Soares MJ, Audus KL. Gestational and smoking effects on peptidase activity in the placenta. Peptides 1998; 19: 1659-1666.

18. Utoguchi N, Magnusson M, Audus KL. Carrier-mediated transport of monocarboxylic acids in BeWo cell monolayers as a model of the human trophoblast. *J. Pharm. Sci.* 1999*;* 88:in press.

19. Utoguchi N, Audus KL. Carrier-Mediated transport of valproic acid in BeWo cells, a human trophoblast cell line. *Int. J. Pharm.* 1999; in press.

20. Patillo RA, Gey GO. The establishment of a cell line of human hormone-synthesizing trophoblast cells in vitro. Cancer Res. 1968; 28: 1231-1237.

21. Huai-Yun H, Secrest DT, Mark KS, Carney D, Brandquist C, Elmquist WF, Miller DW. Expression of multidrug resistance-associated protein (MRP) in brain microvessel endothelial cells. Biochem.Biophys.Res.Commun. 1998; 243: 816-820.

22. Seelig A: A general pattern for substrate recognition by P-glycoprotein. Eur.J.Biochem. 1998; 251: 252-261.

23. Dancis J: Placental Physiology. In: Prenatal and Perinatal Biology and Medicine. Kretchmer N, Quilligan EJ, Johnson JD, eds. Chur, Switzerland: Harwood Academic Publishers, 1987; 1-33.

24. Mylona P, Glazier J, Greenwood S, Sides M, Sibley C: Expression of the cystic fibrosis (CF) and multidrug resistance (MDR1) genes during development and differentiation in the human placenta. Mol.Hum.Reprod. 1996; 2: 693-698.

25. Flens MJ, Zaman GJ, van der Valk P, Izquierdo MA, Schroeijers AB, Scheffer GL, van der Groep P, de Haas M, Meijer CJ, Scheper RJ. Tissue distribution of the multidrug resistance protein. Am. J. Pathol. 1996; 148: 1237-1247.

26. Legrand O, Simonin G, Perrot J, Zittoun R, Marie J: Pgp and MRP activities using calcein-AM are prognostic factors in adult acute myeloid leukemia patients. Blood 1998; 91: 4480-4488.

27. Feller N, Broxterman HJ, Wahrer DCR, Pinedo HM. ATP-dependent efflux of calcein by the multidrug resistance protein (MRP): No inhibition by intracellular glutathione depletion. FEBS Lett. 1995; 368:385-388.

28. Rose JM, Peckham SL, Scism JL, Audus KL: Evaluation of the role of P-glycoprotein in ivermectin uptake by primary cultures of bovine brain microvessel endothelial cells. Neurochem.Res. 1998; 23: 203-209.

29. Kokubu N, Cohen D, Watanabe T: Functional modulation of ATPase of P-glycoprotein by C219, a monoclonal antibody against P-glycoprotein. Biochem. Biophys. Res.Commun. 1997; 230: 398-401.

30. Schinkel AH, Smit JJ, van-Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CAAM, van der Valk MA. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 1994; 77: 491-502.

31. Allikmets R, Schriml LM, Hutchinson A, Romano S, V, Dean M: A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res. 1998; 58: 5337-5339.

32. Sugawara I, Akiyama S, Scheper R, Itoyama S. Lung resistance protein (LRP) expression in human normal tissues in comparison with that of MDR1 and MRP. Cancer Lett. 1997; 112: 23-31.

33. Shapiro AB, Fox K, Lam P, Ling V. Stimulation of P-glycoprotein-mediated drug transport by prazosin and progesterone. Eur. J. Biochem. 1999; 259: 841-850.

Figures Legends

FIG. 1. Calcein-AM (1 µM) accumulation in BeWo cells following exposure to indicated concentrations of cyclosporin A. Data points represent the mean ± SD from at least four different BeWo monolayers. * $p < 0.001$ compared to control .

FIG. 2. Cellular accumulation of ^{3}H-vinblastine in BeWo cells in the presence of cyclosporin A (CSA, 10 µM), quinidine (QUIN, 100 µM), verapamil (VER, 100 µM) or dipyridamole (DPM, 100 µM). Data points represent the mean ± SD from at least four different BeWo monolayers.

* $p < 0.05$, ** $p < 0.001$ compared to control.

FIG. 3. Effects of P-gp inhibitors on calcein-AM or ^{3}H-vinblastine accumulation by primary cultured human trophoblasts. Cells were incubated with calcein-AM (A) or ^{3}H-vinblastine (B) for 30 min in the absence or presence of P-gp inhibitors. CSA; cyclosporin A (10 µM), DPM; dipyridamole (100 µM). Data points represent the mean ± SD from at least four different primary cultures human cytotrophoblasts monolayers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$compared to control.

FIG 4. Immunoprecipitation /Western blot of BeWo cell protein (lane 1) and four different preparations of primary cultures of human cytotrophoblasts (lanes 2-5) generated with the anti-human C219 antibody.

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FIG. 5. Effects of MRP inhibitors on fluorescein accumulation in BeWo cells. BeWo cells were treated with genistein (GEN, 100 µM), valproic acid (VPA, 100 µM), probenecid (PRB, 100 µM) or indomethacin (IND, 10 µM). Data points represent the mean ± SD from at least four different BeWo monolayers.

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Table 1. Effect of P-glycoprotein (P-gp) or multidrug resistance-associated protein (MRP) inhibitors on the accumulation of calcein-AM by BeWo cells.

Target Efflux Mechanism	Efflux Inhibitor	Concentration of Inhibitor (μM)	Relative Accumulation (% of Untreated Control)
P-gp	Cyclosporin A	10	218 ± 46 *
	Quinidine	100	153 ± 40 *
	Verapamil	100	212 ± 35 *
	Dipyridamole	100	215 ± 49 *
MRP	Genistein	100	94 ± 11
	Valproic acid	100	96 ± 4
	Probenecid	100	91 ± 15
	Indomethacin	10	93 ± 10

* raw data significantly different ($p < 0.001$) from calcein-AM uptake in the absence of an inhibitor (Control)

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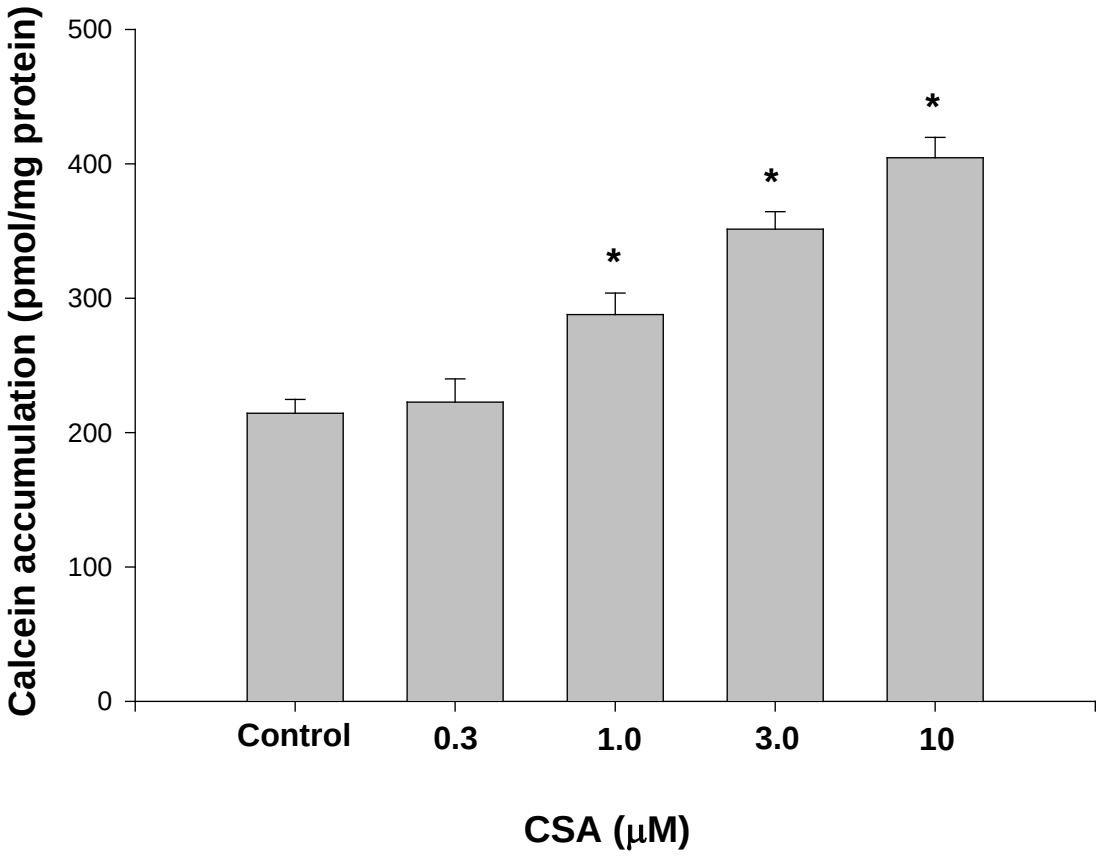
Figure 1: Utoguchi et al.



Utoguchi, N., Chandorkar, G.A., Avery, M., and Audus, K.L. (2000) Functional expression of P-glycoprotein in primary cultures of human cytotrophoblasts and BeWo cells. Reprod. Toxicol. 14, 217-224. PMID: 10838122. Publisher's official version: <http://dx.doi.org/10.1016/S0890-6238(00)00071-X >. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Figure 2: Utoguchi et al.

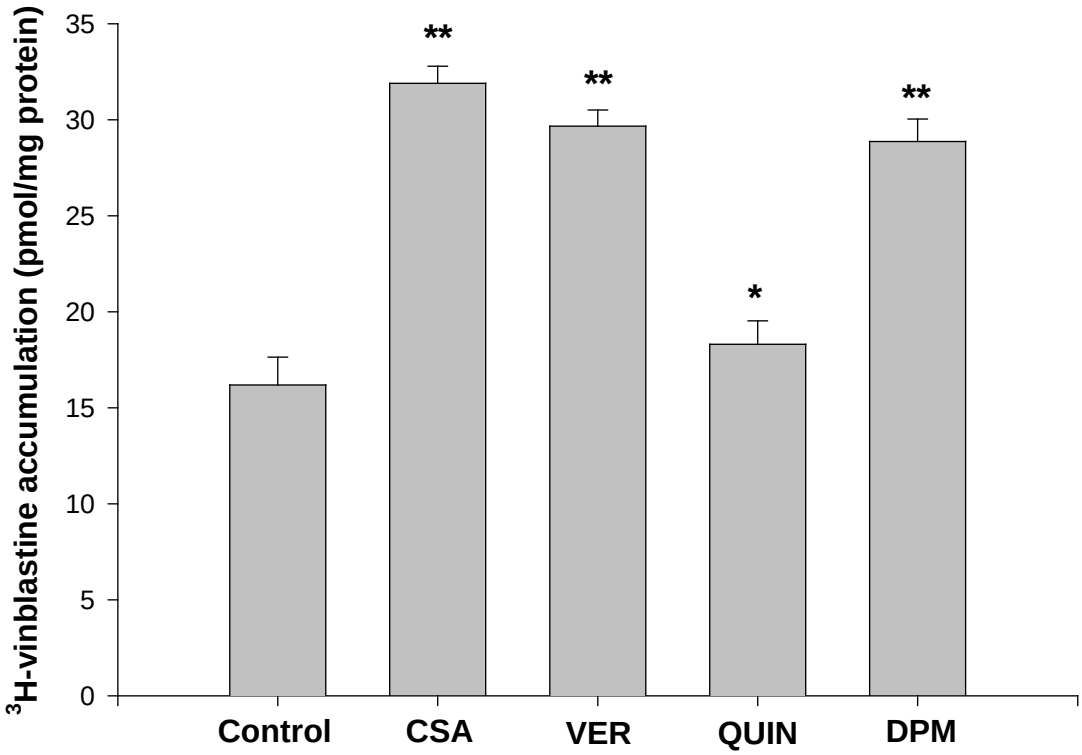
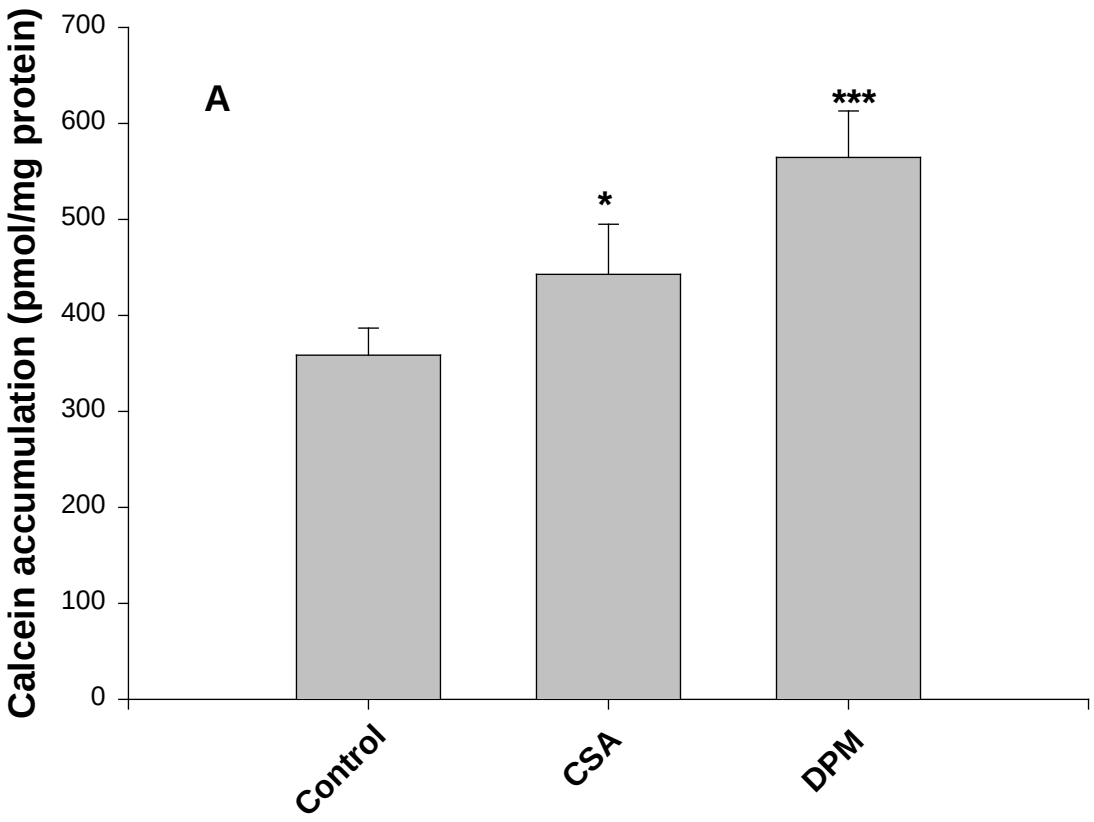
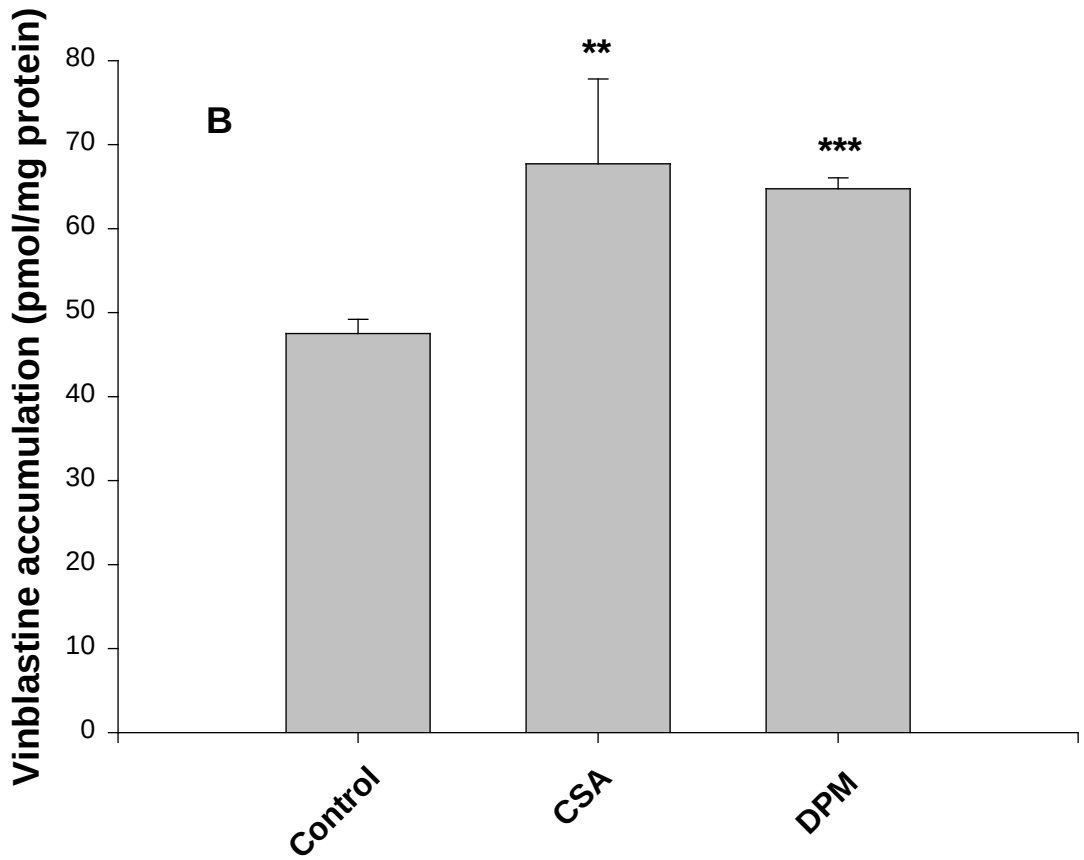


Figure 3: Utoguchi et al.





Utoguchi, N., Chandorkar, G.A., Avery, M., and Audus, K.L. (2000) Functional expression of P-glycoprotein in primary cultures of human cytotrophoblasts and BeWo cells. Reprod. Toxicol. 14, 217-224. PMID: 10838122. Publisher's official version: <http://dx.doi.org/10.1016/S0890-6238(00)00071-X >. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Figure 4: Utoguchi et al.

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Figure 5: Utoguchi et al.