Inhibition of placental P-glycoprotein: impact on indinavir transfer to the foetus

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- We have shown previously using the dually perfused isolated human placenta model that the maternal to foetal transfer of the antiviral protease inhibitor drug indinavir is substantially lower than the transfer in the opposite direction.
- This finding is not consistent with passive diffusion and indicates that a carrier-mediated mechanism is involved in retarding the movement in the maternal to foetal direction.
- The efflux transporter P-gp located in the apical membrane domain of the placental trophoblast cells has been implicated as the likely cause of the differential bi-directional transport.

WHAT THIS STUDY ADDS

- The present study also utilizes the human perfused human isolated placenta to investigate the possible inhibitory effects of the P-gp inhibitor PSC833 and the P-gp substrate/inhibitor ritonavir on the maternal to foetal transfer clearance of indinavir.
- The studies, which were conducted such that each placenta served as its own control, demonstrated a statistically significant increase in the maternal to foetal transfer of indinavir in the presence of PSC833 but not in the presence of ritonavir, a protease inhibitor that is often used in combination with other protease inhibitors in dual therapy.
- The lack of effect of ritonavir is most likely related to the relatively low inhibitory activity at the clinically relevant concentration used in this study.

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Keywords

HIV protease inhibitors, indinavir, P-glycoprotein, placental transfer

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AIMS

To investigate the effect of P-gp inhibition on the maternal to foetal transfer of indinavir.

METHODS

Term human placentae ($n = 12$) were from non-HIV infected women. Maternal to foetal transfer of indinavir was examined in the absence and presence of P-gp inhibitors PSC833 ($n = 7$) or ritonavir ($n = 5$), in the perfused human placenta. Antipyrine and [³H]-vinblastine were included as markers of passive diffusion and P-gp transport, respectively. These markers and indinavir were added to maternal perfusate at 0 min; PSC833 or ritonavir was added at 25 min. Steady-state maternal to foetal transfer clearance was calculated during control and inhibitor phases. Indinavir and vinblastine clearances were normalized to antipyrine clearance (clearance index).

RESULTS

Indinavir clearance index increased between the control (0.25 ± 0.03) and PSC833 phases (0.37 ± 0.14) (95% CI of the difference -0.23 , -0.002). Vinblastine clearance index increased from (0.25 ± 0.08) to (0.34 ± 0.06) in the control and PSC833 phases, respectively (95% CI of difference -0.14 , -0.05). Indinavir clearance index was unchanged between control (0.34 ± 0.14) and ritonavir phases (0.39 ± 0.13) (95% CI of the difference -0.19 , 0.08). Vinblastine clearance index increased from (0.24 ± 0.12) to (0.32 ± 0.12) in the control and ritonavir phases, respectively (95% CI of the difference -0.15 , -0.009).

CONCLUSIONS

Maternal to foetal transfer clearance of indinavir and vinblastine increased following P-gp inhibition. The potential role for co-administration of P-gp inhibitors with PIs to reduce perinatal HIV transmission warrants further investigation.

Introduction

Mother to child transmission of HIV is the principal source of childhood HIV infections [1]. Administration of highly active antiretroviral therapy with protease inhibitors (PIs) during pregnancy, has decreased mother to child transmission of HIV to less than 2% [1]. However, as 17 million women are currently HIV infected [2], it is important to decrease further mother to child transmission of HIV. Anti-retroviral prophylaxis in the foetus is recognized to be important, and therefore preloading the foetus with potent antiretrovirals such as the PIs, may provide valuable protection against mother to child transmission of HIV [3, 4]. For this strategy to be successful, it is essential to understand the placental transfer of PIs and the factors that modulate the transfer.

Although the PIs are routinely used in pregnancy [1], there are limited data on the mechanisms and extent of placental transfer. When PIs are administered during pregnancy, the concentrations in foetal plasma at the time of delivery are low in comparison with the corresponding concentrations in maternal plasma, suggesting a low extent of transfer from mother to foetus [5]. Previous studies in the isolated perfused human placenta revealed low maternal to foetal transfer of ritonavir [6, 7], amprenavir [8] and saquinavir [9, 10], and relatively low transfer of lopinavir [7]. Recently, our group investigated the bidirectional transfer of indinavir in the isolated perfused human placenta [11]. Indinavir transfer was three-fold lower in the maternal to foetal compared with foetal to maternal direction, indicating passive diffusion was not the sole mechanism of placental transfer [11]. Several transport systems, including P-glycoprotein (P-gp), have been identified in placental trophoblast cells [12]. P-gp is strategically localized to the apical (maternal facing) membrane, and active efflux by P-gp appears to restrict the maternal to foetal transfer of xenobiotics [12]. The PIs are substrates for P-gp [13], and the observed differential transfer of indinavir across the placenta [11], is consistent with P-gp limiting indinavir transfer to the foetus.

The ciclosporin analogue, PSC833, is a P-gp inhibitor [13]. Studies in mice revealed co-administration of PSC833 increased the transfer of PIs across the gastrointestinal [14], blood-brain [14, 15], and blood-testis [15] barriers. In a study in pregnant mice, saquinavir concentrations were seven-fold higher in *mdr1a/1b*(-/-) compared with *mdr1a/1b*(+/+) fetuses [3]. In addition, co-administration of PSC833 to pregnant dams increased saquinavir concentrations in *mdr1a/1b*(+/+) fetuses, similar to those observed in *mdr1a/1b*(-/-) fetuses [3]. The PI ritonavir has been shown to inhibit P-gp and increase the uptake of saquinavir in cell culture [16, 17]. Furthermore, Van Praag *et al.* reported that following addition of ritonavir to indinavir therapy in HIV-infected men, concentrations of indinavir increased approximately two-fold in cerebrospinal fluid and eight-fold in seminal plasma, findings that were not fully

explained by increased plasma indinavir concentrations [18]. This is consistent with ritonavir inhibiting P-gp at the blood-brain and blood-testis barriers in humans.

The aim of the present study was to investigate the effect of PSC833 and ritonavir on the maternal to foetal transfer of indinavir in the human isolated perfused placenta; to substantiate the purported role of P-gp in the differential placental transfer of indinavir observed previously in the human placenta [11].

Methods

Materials

Indinavir sulphate was generously donated by Merck Research Laboratories (New Jersey, USA). PSC833 (valsopodar) and ritonavir were provided by Novartis (Basel, Switzerland) and Abbott Laboratories (Illinois, USA), respectively. Analytical grade antipyrine, dextran 70, tetramethylammonium perchlorate, trifluoroacetic acid and verapamil hydrochloride were purchased from Sigma-Aldrich (Castle Hill, Australia). [³H]-Vinblastine (specific activity, 1.85 GBq mmol⁻¹) was from Amersham Biosciences (Castle Hill, Australia), D-glucose from BDH Chemicals (Poole, England) and methanol from Biolab (Melbourne, Australia). All other chemicals were analytical grade or equivalent.

Patients and clinical study protocol

The study protocol was approved by the Ethics Committees of the Royal Women's Hospital and Monash University. Written informed consent was obtained from all participants prior to enrolment. Placentae were obtained from women who had uncomplicated, term pregnancies following normal vaginal deliveries or elective Caesarean sections. Women were excluded if they had HIV/AIDS or any chronic diseases, or were treated with indinavir or other chronic drug therapy. Prior to perfusion, placentae were carefully inspected and those with visible tears were discarded.

Placental perfusion technique

The dual *in vitro* perfusion of the isolated human placenta was adopted from the method of Penfold *et al.* [19], as described previously [11]. Human placentae were perfused using protein-free Krebs-Ringer (Henseleit) solution (pH 7.4) with the addition of 3% dextran and 0.1% glucose [19]. Maternal and foetal flow rates were approximately 14 and 7 ml min⁻¹, respectively. During the initial stabilization period (20 min), the maternal circuit was operated in an open, single-pass mode, while the foetal perfusate was recirculated. The viability of the placenta was assessed during this time by monitoring pressure in the foetal circuit and loss of perfusate from the foetal reservoir. The placental preparation was discarded if there was a change

in pressure (>10 mmHg) or a loss of perfusate (>3 ml h^{-1}). Temperature and pressure were monitored continuously during the perfusion.

Study design

Following the stabilization period, both the maternal and foetal circuits were operated in an open, single-pass mode for the remainder of the perfusion. In each placenta, the maternal to foetal transfer clearance of indinavir was examined in the presence of antipyrine (marker of passive diffusion) [20], and [^3H]-vinblastine (marker of P-gp mediated transport) [21]. A clinically relevant concentration of indinavir (7.60 mg l^{-1}) was added to maternal perfusate, corresponding to plasma unbound concentrations [22]; the concentration of antipyrine was 20.0 mg l^{-1} and vinblastine 0.03 mg l^{-1} (5.00 μCi l^{-1}). In all perfusions, the three analytes were added to the maternal reservoir (0 min); the foetal inflow perfusate was drug-free.

The maternal to foetal transfer of indinavir, vinblastine and antipyrine in the absence and presence of P-gp inhibitors, PSC833 ($n=7$) or ritonavir ($n=5$), was determined. A concentration of PSC833 specific for P-gp inhibition *in vitro* (1.21 mg $\text{l}^{-1} = 1.00$ μM) [23], and a clinically relevant concentration of ritonavir (0.22 mg $\text{l}^{-1} = 0.40$ μM) [24] were selected. The inhibitors were added to the maternal perfusate to mimic the situation *in vivo* in which the P-gp inhibitor would be administered to the mother.

In each placenta, the maternal to foetal transfer of indinavir, vinblastine and antipyrine was examined alone for 25 min (control phase). At the end of the control phase, the P-gp inhibitor (PSC833 or ritonavir) was introduced, and the maternal to foetal transfer of indinavir, vinblastine and antipyrine was examined for a further 25 min (inhibitor phase); hence, each placenta was used as its own control. Maternal and foetal perfusate samples (3 ml) were collected at 3 min intervals to determine the maternal to foetal transfer clearance at steady state during the control and inhibitor phases. Maternal and foetal perfusate samples were stored at -20°C until the time of analysis.

Drug analysis

Perfusate indinavir concentrations were determined using a validated HPLC assay [11]. Calibration standards (0.10 – 10.0 mg l^{-1}) and quality control (QC) samples (0.25 , 1.00 and 10.0 mg l^{-1}) were used in each analytical run. The measured concentrations of the QC samples were within 15% of the nominal concentrations, and reproducibility was within 15% as assessed by coefficients of variation at 1.00 mg l^{-1} and 10.0 mg l^{-1} . For the low QC, the corresponding values were within 20%. The P-gp inhibitors, PSC833 and ritonavir, did not interfere with the chromatography of indinavir or the internal standard, verapamil. Perfusate antipyrine concentrations were determined using a validated HPLC assay [25]. The measured concentrations of the QC samples (0.50 , 5.00 and 20.0 mg l^{-1}) were within 10% of the nominal concentrations and reproducibility was also within 10% for all

QC samples. The chromatography of antipyrine and internal standard, phenacetin, was not influenced by PSC833 or ritonavir. [^3H]-Vinblastine concentrations in perfusate were analyzed using liquid scintillation counting (Packard Tricarb 1900CA scintillation counter).

Pharmacokinetic analysis

The maternal to foetal transfer clearance at steady state ($\text{CL}_{\text{M} \rightarrow \text{F}}$) during the control (19, 22, 25 min) and inhibitor (44, 47, 50 min) phases for indinavir, antipyrine and vinblastine were calculated as described previously [11] (Equation 1).

$$\text{CL}_{\text{M} \rightarrow \text{F}} = \frac{F_{\text{out}} * Q_f}{M_{\text{in}}} \quad (1)$$

where F_{out} is foetal outflow concentration, Q_f is foetal perfusate flow rate, and M_{in} is maternal inflow concentration of the respective analyte. The $\text{CL}_{\text{M}(\text{r})\text{F}}$ -values of indinavir or vinblastine during the control and inhibitor phases were normalized to the respective $\text{CL}_{\text{M}(\text{r})\text{F}}$ of antipyrine, and termed the clearance index (Equation 2).

$$\text{Clearance Index}_{\text{M} \rightarrow \text{F}} = \frac{\text{CL}_{\text{M} \rightarrow \text{F}} \text{ of X}}{\text{CL}_{\text{M} \rightarrow \text{F}} \text{ of Antipyrine}} \quad (2)$$

Statistical analysis

Group data are presented as mean $\pm$ SD. Differences between the maternal to foetal transfer clearance of antipyrine in the first and second 25 min phases, and differences between the maternal to foetal clearance indices in the control and inhibitor phases for indinavir and vinblastine were assessed using the paired Student's *t*-test; $P < 0.05$ was regarded as statistically significant. Statistical analyses were performed using SPSS for Windows version 11.5 (SPSS Inc., Chicago, Illinois).

Results

In preliminary perfusions to assess the viability of placentae over the perfusion duration in the absence of PSC833 or ritonavir, the mean clearance index of indinavir in the first 25 min (0.30 ± 0.11) was not different from that in the following 25 min (0.31 ± 0.13) (95% CI of the difference -0.15 , 0.12 ; $P = 0.49$). The mean clearance index of vinblastine in the first 25 min (0.25 ± 0.13) vs. the following 25 min (0.26 ± 0.18) was also not different (95% CI of the difference -0.39 , 0.37 ; $P = 0.77$). These results indicate the constancy of the maternal to foetal transfer clearances of indinavir and vinblastine, and the viability of the preparation across the 50 min perfusion period.

In the presence of the P-gp inhibitor, PSC833, the foetal outflow concentrations of indinavir and vinblastine increased significantly. The mean clearance index of indinavir increased from the control (0.25 ± 0.03) to PSC833 (0.37 ± 0.14) phases (95% CI of the difference -0.23 , -0.002 ; $P = 0.047$) (Figure 1A). Similarly, vinblastine mean clearance index increased between the control

Figure 1

Clearance indices of (A) indinavir and (B) vinblastine in the control (mean of values at 19, 22 and 25 min) and PSC833 (mean of values at 44, 47 and 50 min) phases. The lines join data from the same placenta ($n = 7$). Mean clearance index of indinavir in the control (0.25 ± 0.03) and PSC833 (0.37 ± 0.14) phases (95% CI of the difference $-0.23, -0.002, P = 0.047$). Mean clearance index of vinblastine in the control (0.25 ± 0.08) and PSC833 (0.34 ± 0.06) phases (95% CI of the difference $-0.14, -0.05, P < 0.003$)

(0.25 ± 0.08) and PSC833 phases (0.34 ± 0.06) (95% CI of the difference $-0.14, -0.05; P < 0.003$) (Figure 1B).

The effect of ritonavir (P-gp inhibitor) on the foetal outflow concentrations of indinavir and vinblastine was also assessed in other placentae. The mean clearance index of indinavir was not significantly different between the control (0.34 ± 0.14) and ritonavir phases (0.39 ± 0.13) (95% CI of the difference $-0.19, 0.08; P = 0.31$) (Figure 2A). In contrast, the mean clearance index for vinblastine increased from the control (0.24 ± 0.12) to ritonavir phases

Figure 2

Clearance indices of (A) indinavir and (B) vinblastine in the control (mean of values at 19, 22 and 25 min) and ritonavir (mean of values at 44, 47 and 50 min) phases. The lines join data from the same placenta ($n = 5$). Mean clearance index of indinavir in the control (0.34 ± 0.14) and ritonavir (0.39 ± 0.13) phases (95% CI of the difference $-0.19, 0.08, P = 0.31$). Mean clearance index of vinblastine in the control (0.24 ± 0.12) and ritonavir (0.32 ± 0.12) phases (95% CI of the difference $-0.15, -0.009, P = 0.04$)

(0.32 ± 0.12) (95% CI of the difference $-0.15, -0.009; P = 0.04$) (Figure 2B).

Discussion

As strategies involving preloading the foetus with PIs have been proposed to reduce mother to child transmission of HIV [3, 4], it is essential to elucidate the mechanisms regulating the placental transfer of PIs. Previously our group demonstrated differential bidirectional transfer of

indinavir (and vinblastine) in the human placenta [11], suggesting potential involvement of placental P-gp in retarding indinavir and vinblastine transfer to the foetus. In the present study, the placental transfer of indinavir and vinblastine was examined in the presence of two P-gp inhibitors, PSC833 and ritonavir [13, 16, 17]. Even though the change in transfer of indinavir and vinblastine was small in some placentae following addition of either P-gp inhibitor, the mean maternal to foetal clearance index of indinavir and vinblastine increased significantly in the presence of PSC833. Similarly, ritonavir significantly enhanced the mean maternal to foetal clearance index of vinblastine, but there was no significant change in the mean clearance index of indinavir. While the number of placentae studied was relatively small, these results indicate P-gp restricts the maternal to foetal transfer of indinavir and vinblastine, consistent with the placenta acting as a pharmacological barrier in humans.

PSC833 significantly increased the maternal to foetal clearance index of indinavir and vinblastine. Ritonavir enhanced the maternal to foetal clearance index of vinblastine; however, there was no significant change in the mean clearance index of indinavir. These results indicate P-gp restricts the maternal to foetal transfer of indinavir and vinblastine, consistent with the placenta acting as a pharmacological barrier in humans.

In the current study, the mean maternal to foetal clearance index of indinavir increased 1.5-fold after addition of PSC833. These results confirm our earlier suggestion that the maternal to foetal transfer of indinavir is modulated by P-gp [11]. Similarly, there is evidence that the placental transfer of another PI, saquinavir, is also modulated by placental P-gp [10]. Using the isolated perfused human placenta model, Molsa *et al.* [10] observed a three-fold higher maternal to foetal transfer of saquinavir in the presence of PSC833. There are several possible reasons for the difference in magnitude of the increase or difference in placental transfer of indinavir (1.5-fold) and saquinavir (three-fold) in the presence of PSC833 in the two studies. Firstly, unlike the present study design, Molsa *et al.* [10] did not use each placenta as its own control to assess possible inhibition of P-gp by PSC833, therefore the results were possibly influenced by interplacenta variability. Secondly, this experimental design in the study by Molsa *et al.* [10] probably contributed to the finding that the bidirectional transfer of antipyrine (marker of passive diffusion) was not equal as expected [11]; the maternal to foetal transfer of antipyrine was six-fold lower than the foetal to maternal transfer [10]. Furthermore, the measurement of placental drug transfer used by Molsa *et al.* [10] was different from that in the current study. Moreover, the concentration of PSC833 chosen by Molsa *et al.* [10] was higher, which may have resulted in more extensive P-gp inhibition. Finally, there is evidence that the two PIs vary in their affinity for P-gp; saquinavir has a higher affinity for P-gp compared with indinavir [26].

In the present study, vinblastine was used in tracer quantities as a marker of P-gp mediated transport [21]. The maternal to foetal transfer of vinblastine increased significantly following inhibition of P-gp with PSC833 or ritonavir. This is consistent with studies in cultured human trophoblast cells which demonstrate vinblastine cell uptake to be significantly enhanced in the presence of various P-gp inhibitors [21, 27]. Ritonavir (1.40 μM) has been shown to inhibit vinblastine transport in cell culture [13]. The current study demonstrated that a clinically relevant concentration of ritonavir (0.40 μM) [24], inhibited vinblastine transport by P-gp, thereby increasing its transfer across the placenta in the maternal to foetal direction.

Ritonavir ($K_m = 0.060 \mu\text{M}$) has a higher affinity for P-gp compared with indinavir ($K_m = 0.47 \mu\text{M}$), as illustrated previously using High Five insect cells expressing human *MDR1* gene [13]. In addition, ritonavir ($\text{IC}_{50} = 3.80 \mu\text{M}$) is a more potent P-gp inhibitor than indinavir ($\text{IC}_{50} = 44.0 \mu\text{M}$) as shown in Caco-2 cells [15]. Nevertheless, ritonavir did not increase the maternal to foetal transfer of indinavir in the present study. This is similar to results in mice in which the placental transfer of saquinavir was not changed in the presence of ritonavir [28]. It is important to note that a clinically relevant concentration of ritonavir was chosen in the current study (0.40 μM) [24], as dual PI therapy with ritonavir is used in pregnancy [1], and hence the results would be relevant for clinical practice. This concentration (0.40 μM) however, was less than the IC_{50} value (3.80 μM) mentioned above [15], and thus there was less likelihood of extensive P-gp inhibition. These results suggest that dual PI therapy with ritonavir is unlikely to achieve a clinically significant increase in indinavir transfer to the foetus.

Previously our group demonstrated the foetal to maternal transfer of indinavir and vinblastine to be two- to three-fold higher than maternal to foetal transfer [11]. In the current study, the mean maternal to foetal transfer of indinavir and vinblastine increased 1.5-fold following addition of the P-gp inhibitor PSC833. There is evidence to suggest that in the presence of P-gp inhibitors, foetal to maternal drug transfer is likely to be reduced [21]. Therefore the transfer of indinavir and vinblastine in each direction across the placenta following P-gp inhibition may be more similar than suggested by comparing data from the bidirectional studies [11] with the present inhibition studies. It is possible that higher concentrations of PSC833 or ritonavir may have achieved more extensive inhibition of placental P-gp, but this was not investigated in the current study as the intention was to use clinically relevant concentrations of the P-gp inhibitors.

In addition to P-gp, other placental transport systems may be involved in modulating the transfer of indinavir and vinblastine. The differential bidirectional transfer of these drugs demonstrated previously [11] suggests involvement of efflux transporters localized to the apical membrane of placental trophoblast cells, such as P-gp, MRP2 and BCRP [12]. Indinavir and vinblastine are sub-

strates for MRP2 [29, 30], but not BCRP [31, 32]. PSC833 and ritonavir may not have inhibited MRP2 in the placenta at the concentrations used in the current study [29, 33], and thus it is possible that MRP2 may be involved in the active efflux of indinavir and vinblastine. However, it is important to note that the level of expression of MRP2 in the placenta is very low [34], hence this transporter is unlikely to contribute significantly to limiting the transfer of these drugs to the foetus. Therefore, the available evidence suggests that P-gp is the predominant transporter mediating the maternal to foetal transfer of indinavir and vinblastine.

In the current study, interplacenta variability was observed in the effect of PSC833 and ritonavir on the transfer clearance of indinavir and vinblastine. The relatively small number of placentae studied precludes an assessment of the full extent of the actual variability. The variability may be due to polymorphisms in the *MDR1* gene encoding for P-gp. Tanabe *et al.* reported the T-129C allele resulted in significantly lower placental P-gp expression [35]. The G2677(A,T) allele was also associated with lower P-gp expression in the placenta, although the results were not statistically significant [35]. Similarly, Hitzl *et al.* observed polymorphisms in the *MDR1* gene at positions 3435 and 2677 were associated with significantly lower placental P-gp expression [36]. Furthermore, P-gp expression was markedly lower when mother and foetus were homozygous for the TT allele at 3435 [36]. Molsa *et al.* investigated the influence of *MDR1* polymorphisms on the placental transfer of saquinavir [10]. The authors concluded the genotype did not affect the PSC833-induced difference in the maternal to foetal transfer of saquinavir, but acknowledged that the number of placentae with various genotypes was low [10]. Recently, increased expression of *MDR1* mRNAs and P-gp in placentae from HIV-1 infected women, compared to noninfected women, has been reported [37]; the relative roles of the infection and maternal administration of antiviral drugs in the former group of women to the increase in placental P-gp was not clear. The implications of heterogeneity in P-gp expression, and induction of P-gp in infected women under treatment, for placental drug transfer require further investigation.

In conclusion, this study confirmed P-gp modulated the maternal to foetal transfer of indinavir in the human placenta. In the presence of the P-gp inhibitor, PSC833, the mean maternal to foetal clearance index of indinavir increased significantly. In comparison, there was no significant change in the mean clearance index of indinavir following addition of a clinically relevant concentration of ritonavir. In the presence of each of the P-gp inhibitors, the change in indinavir transfer was variable; this may have been due to genetic polymorphisms in the *MDR1* gene resulting in variable placental P-gp expression, and warrants further investigation. Thus, the results are consistent with the notion that P-gp mediated efflux creates the placenta as an important pharmacological barrier in humans, thereby creating the foetus as a pharmacological sanctuary.

Co-administration of P-gp inhibitors may be useful in strategies involving preloading the foetus with PIs to decrease mother to child transmission of HIV. Inhibition of placental P-gp, however, is a complex challenge since maternal as well as foetal factors must be taken into consideration, and thus further research into this area is required.

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REFERENCES

- Public Health Service Task Force Perinatal HIVWG. Recommendations for use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in United States. October 12, 2006, 1–65. Available at <http://aidsinfo.nih.gov/ContentFiles/PerinatalGL.pdf> (last accessed 15 September 2007).
- UNAIDS. AIDS epidemic update 2004. Joint United Nations Program on HIV/AIDS, 2004.
- Smit JW, Huisman MT, van Tellingen O, Wiltshire HR, Schinkel AH. Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. *J Clin Invest* 1999; 104: 1441–7.
- Huisman MT, Smit JW, Schinkel AH. Significance of P-glycoprotein for the pharmacology and clinical use of HIV protease inhibitors. *AIDS* 2000; 14: 237–42.
- Pacifici GM. Transfer of antivirals across the human placenta. *Early Hum Dev* 2005; 81: 647–54.
- Casey BM, Bawdon RE. Placental transfer of ritonavir with zidovudine in the *ex vivo* placental perfusion model. *Am J Obstet Gynecol* 1998; 179: 758–61.
- Gavard L, Gil S, Peytavin G, Ceccaldi PF, Ferreira C, Farinotti R, Mandelbrot L. Placental transfer of lopinavir/ritonavir in the *ex vivo* human cotyledon perfusion model. *Am J Obstet Gynecol* 2006; 195: 296–301.
- Bawdon RE. The *ex vivo* human placental transfer of the anti-HIV nucleoside inhibitor abacavir and the protease inhibitor amprenavir. *Infect Dis Obstet Gynecol* 1998; 6: 244–6.
- Forestier F, de Renty P, Peytavin G, Dohin E, Farinotti R, Mandelbrot L. Maternal-fetal transfer of saquinavir studied in the *ex vivo* placental perfusion model. *Am J Obstet Gynecol* 2001; 185: 178–81.
- Molsa M, Heikkinen T, Hakkola J, Hakala K, Wallerman O, Wadelius M, Wadelius C, Laine K. Functional role of P-glycoprotein in the human blood–placental barrier. *Clin Pharmacol Ther* 2005; 78: 123–31.
- Sudhakaran S, Ghabrial H, Nation RL, Kong DC, Gude NM, Angus PW, Rayner CR. Differential bidirectional transfer of

- indinavir in the isolated perfused human placenta. *Antimicrob Agents Chemother* 2005; 49: 1023–8.
- 12 Ganapathy V, Prasad PD. Role of transporters in placental transfer of drugs. *Toxicol Appl Pharmacol* 2005; 207 (Suppl. 2): 381–7.
 - 13 Lee CG, Gottesman MM, Cardarelli CO, Ramachandra M, Jeang KT, Ambudkar SV, Pastan I, Dey S. HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry* 1998; 37: 3594–601.
 - 14 Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM, Wilkinson GR. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest* 1998; 101: 289–94.
 - 15 Choo EF, Leake B, Wandel C, Imamura H, Wood AJ, Wilkinson GR, Kim RB. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug Metab Dispos* 2000; 28: 655–60.
 - 16 Drewe J, Gutmann H, Fricker G, Torok M, Beglinger C, Huwyler J. HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. *Biochem Pharmacol* 1999; 57: 1147–52.
 - 17 Washington CB, Wiltshire HR, Man M, Moy T, Harris SR, Worth E, Weigl P, Liang Z, Hall D, Marriott L, Blaschke TF. The disposition of saquinavir in normal and P-glycoprotein deficient mice, rats, and in cultured cells. *Drug Metab Dispos* 2000; 28: 1058–62.
 - 18 van Praag RM, Weverling GJ, Portegies P, Jurriaans S, Zhou XJ, Turner-Foisy ML, Sommadossi JP, Burger DM, Lange JM, Hoetelmans RM, Prins JM. Enhanced penetration of indinavir in cerebrospinal fluid and semen after the addition of low-dose ritonavir. *AIDS* 2000; 14: 1187–94.
 - 19 Penfold P, Drury L, Simmonds R, Hytten FE. Studies of a single placental cotyledon *in vitro*. I. The preparation and its viability. *Placenta* 1981; 2: 149–54.
 - 20 Schneider H, Progler M, Sodha RJ. Effect of flow rate ratio on the diffusion of antipyrine and 3H₂O in the isolated dually *in vitro* perfused lobe of the human placenta. *Contrib Gynecol Obstet* 1985; 13: 114–23.
 - 21 Ushigome F, Takanaga H, Matsuo H, Yanai S, Tsukimori K, Nakano H, Uchiumi T, Nakamura T, Kuwano M, Ohtani H, Sawada Y. Human placental transport of vinblastine, vincristine, digoxin and progesterone: contribution P-Glycoprotein. *Eur J Pharmacol* 2000; 408: 1–10.
 - 22 Csajka C, Marzolini C, Fattinger K, Decosterd LA, Telenti A, Biollaz J, Buclin T. Population pharmacokinetics of indinavir in patients infected with human immunodeficiency virus. *Antimicrob Agents Chemother* 2004; 48: 3226–32.
 - 23 Dorr R, Karanes C, Spier C, Grogan T, Greer J, Moore J, Weinberger B, Schiller G, Pearce T, Litchman M, Dalton W, Roe D, List AF. Phase I/II study of the P-glycoprotein modulator PSC 833 in patients with acute myeloid leukemia. *J Clin Oncol* 2001; 19: 1589–99.
 - 24 Abbott Laboratories. North Chicago, Illinois, Norvir® Product Information, May 2007.
 - 25 Ghabrial H, Czuba MA, Stead CK, Smallwood RA, Morgan DJ. Transfer of acipimox across the isolated perfused human placenta. *Placenta* 1991; 12: 653–61.
 - 26 Bachmeier CJ, Spitzenberger TJ, Elmquist WF, Miller DW. Quantitative assessment of HIV-1 protease inhibitor interactions with drug efflux transporters in the blood–brain barrier. *Pharm Res* 2005; 22: 1259–68.
 - 27 Utoguchi N, Chandorkar GA, Avery M, Audus KL. Functional expression of P-glycoprotein in primary cultures of human cytotrophoblasts and BeWo cells. *Reprod Toxicol* 2000; 14: 217–24.
 - 28 Huisman MT, Smit JW, Wiltshire HR, Hoetelmans RM, Beijnen JH, Schinkel AH. P-glycoprotein limits oral availability, brain, and fetal penetration of saquinavir even with high doses of ritonavir. *Mol Pharmacol* 2001; 59: 806–13.
 - 29 Huisman MT, Smit JW, Crommentuyn KM, Zelcer N, Wiltshire HR, Beijnen JH, Schinkel AH. Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. *Aids* 2002; 16: 2295–301.
 - 30 Huisman MT, Chhatta AA, van Tellingen O, Beijnen JH, Schinkel AH. MRP2 (ABCC2) transports taxanes and confers paclitaxel resistance and both processes are stimulated by probenecid. *Int J Cancer* 2005; 116: 824–9.
 - 31 Gupta A, Zhang Y, Unadkat JD, Mao Q. HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2). *J Pharmacol Exp Ther* 2004; 310: 334–41.
 - 32 Litman T, Brangi M, Hudson E, Fetsch P, Abati A, Ross DD, Miyake K, Resau JH, Bates SE. The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). *J Cell Sci* 2000; 113: 2011–21.
 - 33 Davey MW, Hargrave RM, Davey RA. Comparison of drug accumulation in P-glycoprotein-expressing and MRP-expressing human leukaemia cells. *Leuk Res* 1996; 20: 657–64.
 - 34 Pascolo L, Ferneti C, Pirulli D, Crovella S, Amoroso A, Tiribelli C. Effects of maturation on RNA transcription and protein expression of four MRP genes in human placenta and in BeWo cells. *Biochem Biophys Res Commun* 2003; 303: 259–65.
 - 35 Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, Otsubo K. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR) -1 gene. *J Pharmacol Exp Ther* 2001; 297: 1137–43.
 - 36 Hitzl M, Schaeffeler E, Hochar B, Slowinski T, Halle H, Eichelbaum M, Kaufmann P, Fritz P, Fromm MF, Schwab M. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). *Pharmacogenetics* 2004; 14: 309–18.
 - 37 Camus M, Delomenie C, Didier N, Faye A, Gil S, Dauge MC, Mabondzo A, Farinotti R. Increased expression of MDR1 mRNAs and P-glycoprotein in placentas from HIV-1 infected women. *Placenta* 2006; 27: 699–706.