The use of antiretroviral drugs has allowed for a spectacular reduction in mother-to-child transmission (MTCT) of HIV in the industrialized countries, with a current rate of ≤0.5% in France (1, 2). Darunavir is an HIV protease inhibitor (PI) that is increasingly used in combination with other drugs to treat HIV infection. It is now considered a first-line option in pregnant women (3, 4), although few data are available to date on its effects in utero (9), particularly mitochondrial disease (6) and hematologic or cardiac function toxicities (7), whereas few antiretroviral drugs have shown any relation to the risk of malformations (8). The HIV protease inhibitors are largely well tolerated in utero (9), although there are reports of elevated neonatal bilirubin levels following atazanavir exposure (10) and transient adrenal dysfunction following perinatal lopinavir-ritonavir treatment (11). Determining fetal exposure to a specific drug is important in estimating its potential for preexposure prophylaxis (12), as well as its risk for toxicities in the fetus. Since data from animal studies are difficult to extrapolate to humans due to the differences in placental physiology, human studies are required. There are some data on cord blood concentrations of darunavir at delivery, but these data reflect only a single time point, and larger series are required for population pharmacokinetic modeling (13, 14). The ex vivo human cotyledon is an accepted model in which to study and interpret placental transfer (15).

The purpose of this study was to investigate the placental transfer of darunavir in the ex vivo human perfused cotyledon.

Placentas were collected after uneventful pregnancies and term deliveries (≥37 weeks gestational age) in a single center (a university hospital maternity department in Colombes, France) and were rapidly perfused on site. Written informed consent was obtained from each woman who donated a placenta, according to French bioethics guidelines (article L1211-2 of the Public Health Code).

The darunavir base was provided by the manufacturer (Janssen, Issy-les-Moulineaux, France) as antipyrine–phosphate-buffered saline (PBS). Bradford reagent was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France), other reagents from Invitrogen (Cergy Pontoise, France), and albumin from Baxter Laboratories (Deerfield, IL).

Placental transfer of the HIV protease inhibitor darunavir was investigated in 5 term human cotyledons perfused with darunavir (1,000 ng/ml) in the maternal to fetal direction. The mean (± the standard deviation [SD]) fetal transfer rate (FTR) (fetal/maternal concentration at steady state from 30 to 90 min) was 15.0% ± 2.1%, and the mean (±SD) clearance index (darunavir FTR/antipyrine FTR) was 40.3% ± 5.8%. This shows that darunavir crosses the placenta at a relatively low rate, resulting in fetal exposure.
described (19). The lower limit of quantification for darunavir was 5 ng/ml. Antipyrine concentrations were determined by high-performance liquid chromatography with UV detection at 290 nm after liquid-liquid extraction. The analytic column for antipyrine separation consisted of an octadecylsilyl NovaPak (3.9 mm by 150 mm). The mobile phase comprised 0.05 M phosphate buffer (pH 3)-methanol-tetrahydrofuran (75:25:0.9 [vol/vol/vol]). Standard curves for antipyrine concentrations ranged from 0.5 to 20 mg/liter. The lower limit of quantification for antipyrine was 0.01 mg/liter.

Maternal-to-fetal transfer parameters were calculated at steady state according to the formulas of Challier et al. (20). The fetal transfer rate (FTR) is the ratio of fetal to maternal concentrations, and the clearance index (CLI) is the ratio of the FTR of darunavir over the FTR of antipyrine. An antipyrine FTR of >20% was required to validate each experiment.

Eight full-term placentas were donated by mothers who were seronegative for HIV and hepatitis B and C and who had taken no medication, except for vitamin supplements and perimedullar analgesia. Of eight placentas that were perfused, five procedures were validated, with a mean (± standard deviation [SD]) antipyrine FTR of 35.8% ± 6.6%. The results of the five experiments at steady state are summarized in Table 1.

The mean (±SD) concentration in the maternal compartment was 903 ± 308 ng/ml, about 16 times the 50% effective concentration (EC_{50}) against wild-type HIV (55 ng/ml, corrected for plasma protein binding) (21). The mean concentration in the fetal compartment was 132 ± 32 ng/ml, which is still well above this EC_{50}.

The mean (±SD) FTR of darunavir was 15.2% ± 3.2%, and the mean (±SD) clearance index (CLI) was 35.6% ± 5.0%.

There was a fairly good correlation between the maternal and fetal concentrations (coefficient of correlation, 0.81). This study indicates that about one-sixth of maternal darunavir is transferred across the human placenta. The mean trough level in the fetal compartment was 140 ng/ml, near the suggested levels for darunavir use in pregnant women (22). Clinical data on darunavir use in pregnant women are summarized in Table 1.

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There was a fairly good correlation between the maternal and fetal concentrations (coefficient of correlation, 0.81).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Placental transfer of darunavir in ex vivo perfused human cotyledons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>Darunavir concentration</td>
</tr>
<tr>
<td>1</td>
<td>897</td>
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<tr>
<td>2</td>
<td>897</td>
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<tr>
<td>3</td>
<td>897</td>
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<td>4</td>
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<td>5</td>
<td>897</td>
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</table>

Data are from five experiments. M, maternal; F, fetal; CLI, clearance index.

As for darunavir in the present study, the ex vivo placental perfusion results were consistent with in vivo data for other protease inhibitors. For lopinavir combined with ritonavir, we previously reported (31) a mean (±SD) FTR of 23.6% ± 6.9% and a mean (±SD) clearance index of 73% ± 16% at an albumin concentration of 2 g/liter. The fetal transfer rates decreased at higher albumin concentrations. For nelfinavir, the mean (±SD) FTR was 14% ± 3.4% and the mean (±SD) clearance index was 39% ± 10% (15). For saquinavir, there was nearly no placental transfer (17).
Interestingly, among the protease inhibitors, those which are more lipophilic than darunavir and highly protein bound have lower placental transfer rates (17, 31). The protein binding rate for darunavir is 95%, which is considerably less than that for lopinavir or ritonavir and somewhat more than that for atazanavir (26). Darunavir is a substrate of efflux transporters expressed in the placenta, as are the HIV protease inhibitors, in general (32), and some other antiretroviral drugs (18), resulting in decreased fetal exposure (33).

The results obtained with the ex vivo placental perfusion model have already been validated for various antiretroviral agents (15). The main limitation is that the model evaluates placental transfer at term and not during the entire pregnancy. The same is true for clinical cord blood data at delivery. Although the experiments do not entirely reproduce in vivo conditions, the procedure is carefully monitored and standardized to approach normal physiology, and the FTR of antipyrine is monitored in order to control the integrity of the placental barrier.

The maternal plasma concentration decreases for darunavir, as for other protease inhibitors, in the third trimester of pregnancy (23, 26), although Zorrilla et al. (26) observed that the plasma concentrations of unbound darunavir do not decrease. Twice-daily dosing is now recommended during pregnancy (3). The darunavir concentrations that we perfused in the maternal compartment were below the mean trough plasma concentration reported in late pregnancy, which was 1,407 ng/ml for once-daily darunavir (800 mg) plus ritonavir (100 mg) (24). In clinical studies of cord/maternal concentrations at delivery (23), maternal concentrations were widely dispersed, reflecting the variable timing between the last dose taken and the delivery. We chose not to add ritonavir to darunavir in the experiments because it was not required to increase the bioavailability, since the drug is added directly into the maternal perfusate and because there is little placental transfer of ritonavir and no accumulation in the placenta (34).

In conclusion, the placental transfer of darunavir appears to be limited, on the same order as that of most of the other protease inhibitors, but sufficient to expose the fetus to drug concentrations near the therapeutic range. This may have some effect as preexposure prophylaxis (PrEP), but it also carries a potential for adverse effects. Furthermore, protease inhibitors may accumulate in the fetus during gestation, as has been suggested from studies using neonatal hair samples to determine lopinavir and ritonavir concentrations (35). Clinical studies are required to determine whether darunavir has a more favorable safety and efficacy profile than those of other protease inhibitors for use in pregnant women.

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REFERENCES
5. Reference deleted.


