**Therapeutic drug monitoring of darunavir/ritonavir in pregnancy**

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Background

Physiological changes during pregnancy can have a significant impact on antiretroviral pharmacokinetics (PK), which may result in reduced drug efficacy. Ensuring optimal plasma concentrations of antiretrovirals is essential for maternal health and prevention of mother-to-child transmission (PMTCT). Here we describe darunavir/ritonavir (DRV/r) PK in a cohort of pregnant women undergoing routine TDM as well as the transplacental passage of DRV by measuring and comparing cord blood and maternal blood samples post-delivery.

Methods

Pregnant HIV-positive women received DRV/r as part of routine pre-natal care. Demographic and clinical data were collected. DRV plasma concentrations [DRV] were determined in the first (T1), second (T2) and third (T3) trimester and at postpartum (PP) using HPLC-MS/MS methodology (Lab21, Cambridge UK). Where possible paired maternal and cord blood samples were taken at delivery.

Results

33 women (22 Black African, 11 Caucasian) were enrolled. All received DRV/r at a dose of 800mg/100mg OD over the course of pregnancy and postpartum. 15 received ART prior to pregnancy and 18 initiated DRV/r in pregnancy. At the time nearest to delivery, all but 4 had undetectable plasma viral loads (pVL). [DRV] were determined in 1 (T1); 14 (T2); 32 (T3) and 29 (PP). [DRV] were significantly lower at T2/T3 relative to PP.

Conclusion

[DRV] in T3 were 14% lower compared to T2 and [DRV] in T2 and T3 were only 36-55% when compared to PP. However, DRV pharmacokinetics in pregnancy were not associated with a lack of virologic suppression at delivery as of the 33 patients enrolled in this study, 31 had no HIV transmission from mother to child. Data regarding two of the candidates were not available as they delivered in a separate healthcare facility. Nevertheless, careful monitoring of HIV patients in pregnancy is required to ensure successful viral suppression and PMTCT.

**Introduction**

Combination antiretroviral therapy (cART) is the recommended treatment for HIV in pregnancy and the prevention of mother to child transmission (1,2).

Pregnancy is a state associated with significant alteration to drug absorption, distribution, metabolism and elimination, which can affect the efficacy of antiretroviral therapy for both mother and child (1,2). Hence the study of the pharmacokinetics of antiretroviral therapy is vital if optimal plasma concentration of antiretroviral drugs in pregnancy and the minimising of risk of vertical transmission of HIV is to be achieved.

Darunavir (DRV) is the second generation of the protease inhibitor class of anti-retrovirals which act by inhibiting HIV-1 protease, which catalyses the formation of an infective HIV virion (3). It was first approved by the FDA on 23rd June 2006 (4) and first approved for treatment in pregnant women in June 2016 (5).

In June 2016, the FDA updated their product label info for DRV marketed under the trade name of Prezista. Updated information included a dosage recommendation for pregnant women. Recommended dosage was DRV 600mg with ritonavir 100mg taken twice daily, with consideration for the dosage of DRV 800mg and ritonavir 100mg once daily in patients who already on such a dose prior to pregnancy, or in those patients in whom a twice daily dose may lead to non-compliance (6).The dosage of DRV/ritonavir (DRV/r) prescribed in this paper is in keeping with the latest FDA recommendations. DRV/r 800mg/100mg has been demonstrated to be a viable treatment option in pregnant women (7).Trials have demonstrated that there is statistically no significant difference in terms of changes of CD4 cell count when comparing DRV/r 800/100 once daily and DRV/r 600/100mg twice daily in the general adult population (8).

DRV has been classified by the FDA as Pregnancy Class C. Animal trials involving oral gavage administration of DRV to rats and mice up to 104 weeks have shown a dose-related increase in the incidence of hepatocellular adenomas and carcinomas in both species. Additionally, there was a noted increased incidence of thyroid follicular cell adenomas in male rats. Repeated delivery of DRV has been observed to predispose rats (but not humans) to thyroid neoplasms. DRV was not noted to be genotoxic or mutagenic in mice or humans (5).

DRV must be administered with ritonavir as low dose ritonavir serves to prolong the half-life of darunavir (5,8,9).

To date, studies published regarding the efficacy of DRV/r treatment in pregnancy and postpartum are based on case reports and retrospective data collection (7,10–15). Literature surrounding this topic explore the efficacy of alternative doses of DRV/r 600mg/100mg BD VS DRV/r 800mg/100mg OD (7,10–16). Several studies compare the umbilical cord blood concentration of DRV to maternal blood concentration (15,17).

The aim of this paper was to study the pharmacokinetic profile, antiviral activity and safety of DRV/r 800mg/100mg OD in HIV infected pregnant women.

**Methods**

***Subjects***

HIV-infected pregnant women attending the Rotunda Hospital were recruited to be part of this study. Informed consent was obtained from the patients to receive a triple drug ART regimen containing the oral DRV capsule boosted with ritonavir at a standard dose of DRV/r 800/100mg OD as part of their antiretroviral regimen, have their plasma DRV concentrations measured throughout pregnancy and for the data to be published in this study.

***Study Design***

Blood sampling was performed in the first, second and third trimesters as well as in the postpartum period in women on antiretroviral treatment including DRV/r. Maternal and cord blood samples were also taken at labour and delivery. Demographic and clinical parameters were corrected. Plasma Viral Load (pVL) of HIV and CD4 cell counts were monitored at the time of TDM sampling and at the time nearest to delivery.

***Analytical and Pharmacokinetic Methods***

Blood samples were taken by venipuncture the morning after the previous dose of DRV/r (approximately 14-20 hours post-dose). The time of drug intake was recorded. In addition, maternal blood and umbilical cord blood samples were collected at delivery.

Blood was collected in heparin tubes and centrifuged at 1000 *g*, 10 min; 4˚C and the plasma removed and stored at -30˚C. Prior to analysis the plasma was heat inactivated (58˚C, 40 min). Total plasma DRV concentrations were determined using a validated HPLC-MS/MS methodology (Lab21, Cambridge UK) (18). The assay lower limit of quantification (LLQ) for DRV was 78.1 ng/mL.

***Data Analysis***

The demographic and clinical characteristics are presented as the median (range). Measured DRV plasma concentrations were expressed as the geometric mean with 95% confidence intervals (95% CI). The measured concentrations were then extrapolated to 24 hours post-dose (C24; ng/mL) using the established half-life (15 hours) for DRV in the presence of RTV (Prezista 400 mg film coated tablets, Summary of Product Characteristics). This enabled normalisation of the effect of variable sampling times post drug intake. Values with missing drug intake data were excluded. Inter-subject variation in plasma concentrations was estimated using a coefficient of variation, expressed as a percentage (CV%) [%CV = (standard deviation/mean)\*100]. DRV plasma concentrations at antepartum and postpartum were related to the drug’s EC50 (550 ng/mL) for protease inhibitor resistant HIV-1 strains (5). The placental transfer of DRV was determined by calculation of a cord blood-to-maternal plasma ratio (C/M ratio) from maternal and cord blood samples obtained at delivery.

Differences antepartum versus postpartum were assessed independently using a One-Way ANOVA, with a Bonferroni correction to test for multiple comparisons. Normality of data was assessed using a Shapiro-Wilk test, and non-normally distributed data were log transformed. All statistics were performed and analysed using Statsdirect (Version 3.0.171©, Biomedical Software, Statsdirect Ltd, Cheshire, UK). P values were two-sided at the 0.05 significance level.

***Ethical review***

Ethical approval was granted by the Rotunda Hospital’s research ethics committee.

**Results**

A total of 33 women were enrolled on the study (22 Black African, 11 Caucasian). A third of the patients (11/33) were newly diagnosed at the antenatal clinic. Patient baseline characteristics are summarised in Table 1. Fifteen women were receiving ART prior to pregnancy. 18 women (11 treatment naive, 7 experienced) initiated DRV/r therapy in pregnancy. All took therapy for at least 13 weeks prior to the first TDM sampling. The median (range) gestational age at the time of DRV/r treatment initiation in these patients was 19 weeks (13-24). All patients were prescribed DRV/r at the standard dose of 800/100 mg once daily. The NRTI backbone was primarily emtricitabine + tenofovir (Truvada) in 28 (85%) patients.

DRV concentrations were determined in 1 patient in the first trimester, 14 patients in the second and 32 patients in the third trimester. The patient that underwent pharmacokinetic sampling in the first trimester had her TDM taken at 13 weeks. Median (range) gestational age at the time of pharmacokinetic sampling was 21.5 weeks (20-24) in the second trimester and 30 weeks (25-39) in the third trimester, respectively. In addition, 29 patients had measurements taken at postpartum with A median follow up time after delivery of 8 weeks (2-57).

Geometric mean (95% CI) DRV concentrations were marginally different between the second (1401 ng/mL, 1041-1760) and third trimesters (1201 ng/mL, 910-1493) showing a 14% reduction in the third trimester (p=0.422). However, DRV concentrations during the second and third trimesters were both significantly lower (46-55%) than the postpartum period (2588 ng/mL, 1655-3521) (p<0.003). Normalisation of measured DRV plasma concentrations to a 24 hour trough concentration resulted in a 49-54% decrease at antepartum compared with postpartum (p<0.003), with only a 10% difference between the second and third trimesters (p=0.615)

The difference in the total number of patients who had TDM undertaken in the second trimester (n=14), the third trimester (n=32) and postpartum (n=29), indicate the possibility of inaccuracy due to different cohort sample sizes. If we consider the only the plasma concentrations of patients who had TDM sampling in both the second and third trimester and postpartum (n=10), then we see a difference of 12% between the second (1463 ng/mL, 982-1945) and third (1289 ng/mL, 842-1736) trimesters (p=0.585), and a 48-53% difference between antepartum and postpartum (2802 ng/mL, 1847-3756) (p<0.009) Following normalisation there was a 45-53% decrease in 24 hour trough concentrations antepartum compared with postpartum (p<0.015), and a 15% difference between the second and third trimesters, respectively (p=0.474).

Inter-subject variation in DRV plasma concentrations was moderately high throughout pregnancy (49-69%) and increased at postpartum (94%). The median time of sampling (post-dose) was consistent during the second and third trimesters of pregnancy and postpartum, at approximately 18-20 hours (p=0.510).

5 of 33 (15%) patients had DRV concentrations below the proposed DRV MEC (<550 ng/ml) in third trimester with one undetectable reading. Only one of the four patients had her DRV concentration checked during the second semester, and it was noted to be above 550ng/ml then. However, one of the five patients had sustained swine flu during the third trimester testing which may have affected compliance to medication. All five achieved undetectable pVL based on the VL closest to delivery. At postpartum, one patient had DRV TDM <550ng/ml, two had undetectable readings and two were unavailable. One of the patients with undetectable readings admitted to reduced compliance to medication due to forgetfulness. The one patient with a DRV TDM <550ng/ml had a TDM of 346 during the third trimester.

A total of 18 patients had paired maternal and umbilical cord blood samples taken at delivery to ascertain DRV placental transfer. Out of the 18 maternal samples taken, 15 of them were taken before delivery (Median time of 5.13 hours, range: 1.00 – 17.45 hours), two were taken after delivery (Median time of 3.37 hours, range: 1.65-5.1 hours), and one was taken at delivery. All cord blood samples were obtained minutes after delivery. DRV plasma concentrations in maternal and cord samples at the time of delivery are depicted in Figure 1. The median (range) time from the last DRV dose and collection of maternal and cord blood at delivery was 11 hours (1.9-36) and 15 hours (1.9-44), respectively. DRV was detectable in all maternal plasma samples, and in 17/18 cord plasma samples. For one subject with undetectable DRV in cord plasma (<78 ng/mL), the corresponding maternal plasma concentration was 607 ng/mL. The geometric mean (95% CI) DRV concentrations were higher in maternal plasma (1878 ng/ml, 1172-2584) than cord plasma (309ng/ml, 0.3-618) in 16 of 18 patients with paired samples. The median C/M ratio was 0.11 (0.06-2.46), respectively. See Table 2.

At the time nearest to delivery 28 patients had undetectable pVL, 4 patients had detectable pVL [77, 91, 114, 176 copies/mL] and 1 was unavailable. Although, TDM sampling in these subjects was limited, 2/4 patients had therapeutic concentrations in the second trimester with the other two not having a TDM. In the third trimester 3 out of 4 patients had therapeutic concentrations and one patient had subtherapeutic levels. A single subject had undetectable plasma concentrations in the third trimester, which suggested issues with compliance to medication. There were 33 livebirths with one death occurring later due to baby being born Trisomy 13. Two patients (6%) delivered in another maternity unit and so mode of delivery was unavailable. Of the remaining 31 women, 14 (45%) were born by spontaneous vaginal delivery (SVD), 17 (55%) were by caesarean section (8 elective; 9 emergency). The median gestational age at delivery was 39.6 weeks (30.0-41.6 weeks) and median birthweight was 3.16kg (1.76-3.89kg). There were no cases of vertical transmission in the cohort.

**Discussion**

The treatment progress and results on a total of 33 patients from the Rotunda Hospital, Ireland are studied in this paper. The 33 patients involved in the study all received once daily dosing of DRV/r 800mg/100mg during pregnancy. In addition, 18 of the 33 cases had maternal and cord blood concentrations of DRV taken at delivery.

Current papers studying pharmacokinetics of DRV in pregnancy have reported a common trend where plasma concentrations of DRV progressively drop from the 1st to the 3rd trimester, then showing a rise postpartum. This trend is noted in both twice daily (BD) dosing of DRV/r 600mg/100mg and once daily (OD) dosing of DRV/r 800mg/100mg (2,6,7,10–14,16). Our study reflects this trend with reductions observed as pregnancy progressed and statistically significant reductions in DRV concentration observed between the antenatal and postnatal period.

An objective of our study was to determine the validity of current American guidelines, which recommend DRV/r 600mg/100mg BD in pregnant woman with HIV-1. Pregnant women with HIV-1 infections in our study receiving once daily DRV/r 800mg/100mg, exhibited median plasma DRV concentrations of 3790ng/mL, 1401ng/mL, 1201ng/mL and 2588ng/mL in the first, second, third trimesters and the postpartum period respectively. However, it should be noted data from the first trimester is representative of a single patient. DRV EC50 is 55ng/mL for wild type virus (19) and 550ng/mL for resistant virus strains (5). Our study notes that in the majority of our patients, plasma Cmax remain above 550ng/mL throughout pregnancy and in postpartum. This supports claims that DRV/r 800mg/100mg taken once daily is sufficient dosing for pregnant women with HIV-1 infections. This study is unfortunately limited by the lack of patient data obtained in the earlier trimesters and hence a study with more data would be warranted to fully support this claim.

Aims of treatment in HIV-1 infected women in pregnancy include viral suppression in the pregnant mother as well as prevention of mother to child transmission. Mother to child transmission of HIV is believed to occur primarily during birth when the foetus is exposed to maternal blood and genital secretions. Hence viral suppression is of vital importance. Having said that, there have been documented cases, albeit rare ones, where perinatal transmission has occurred despite undetectable or low levels of HIV RNA in pregnant mothers (20–22).Such cases suggest that viral suppression in pregnant mothers alone is insufficient and that vertical transfer of ART from mother to child via the placenta to provide peripartum prophylaxis is another important factor which may require monitoring.

Measuring drug concentrations in blood samples taken from the umbilical cord post-delivery give us a snapshot estimate at how well placental transfer of DRV has occurred. The obvious flaw with this method of measurement is that a spot measurement of maternal and umbilical cord concentrations of DRV may not necessarily portray concentration trends throughout the entire pregnancy.

Protease inhibitors such as DRV are documented to have a relatively low degree of transplacental transfer and is highly variable between different individuals (2,22–24). Foetal DRV concentrations are the result of multiple factors, main ones including maternal plasma concentration, placental blood flow, permeability of placenta and the rate of foetal elimination of darunavir. 24 As the factors are subject to change throughout pregnancy, it is challenging to determine the transplacental passage of DRV throughout pregnancy.

Our study observes the ability of the DRV/r 800mg/100mg once daily dosing at providing peripartum prophylaxis by comparing cord levels obtained from our patients and comparing them to documented and published EC50 Levels. Janssen quotes an *in vitro* EC50 of 1.2 to 8.5 nM (0.7 to 4.7 ng/ml)(25)..Our study data shows cord blood levels more than ten-fold of the quoted values, which should correlate with good *in vitro* viral suppression. This is reflected by the results of our study. Apart from two cases with uncertain outcome as the patients delivered in another medical facility, there were no cases of mother to child transmission detected.

**Conclusion**

The data collected from our study show that good results can be expected from a once daily regime of DRV/r 800mg/100mg. This is measured by the DRV plasma concentration at different stages of pregnancy, viral suppression and prevention of mother to child transmission of disease.

There were a small number of cases in our study where treatment failure had resulted in reduced levels of DRV concentration and unsuppressed viral loads. But in the majority of these cases had complications namely interruption to treatment due to non-compliance and co-infections which may have affected the outcome of the treatment. Aside from two cases where delivery took place in another medical facility, there were no detected transmissions of HIV from mother to child.

The results of our study show that once daily dosing of DRV/r 800mg/100mg provided sufficient antiretroviral therapy with good efficacy and viral suppression as well as peripartum prophylaxis. Unfortunately, the data obtained from our study is incomplete, especially in the early phase of pregnancy and the cord blood samples. Hence further studies should be conducted to confirm the conclusion above.

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