A quest to optimize the clinical pharmacology of tuberculosis and human immunodeficiency virus drug treatment

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Risk factors contributing to a low darunavir plasma concentration

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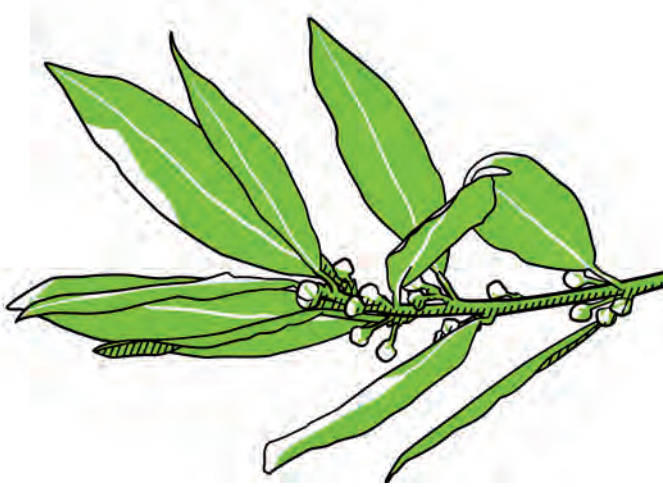

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Summary

Darunavir is an efficacious drug, however pharmacokinetic variability has been reported. The objective of this study was to find predisposing factors for low darunavir plasma concentrations in patients starting the once- or twice-daily dosage. Darunavir plasma concentrations from January 2010 till December 2014 of HIV-infected individuals treated in the outpatient clinic of the University Medical Center Groningen were retrospectively reviewed. The first darunavir plasma concentration of patients within 8 weeks after initiation of darunavir therapy was selected. A dichotomal logistic regression analysis was conducted to select the set of variables best predicting a darunavir concentration below median population pharmacokinetic curve. In total 113 patients were included. The variables best predicting a darunavir concentration besides food intake included age together with estimated glomerular filtration rate (Hosmer and Lemeshow Test p=0.945, Nagelkerke R Square 0.284). Systematic evaluation of TDM results may help to identify patients at risk for low drug exposure.

Introduction

Darunavir (DRV) is a human immunodeficiency virus (HIV) protease inhibitor (PI) which is used for the treatment of HIV-1 infection in antiretroviral treatment-naïve and treatment-experienced adults and paediatric patients aged ≥6 years [1]. Once-daily DRV 800 mg is approved for use in treatment-naïve patients and the twice daily dosage (bid) of DRV 600 mg is approved for use in treatment-experienced adults with DRV resistance-associated mutations [2]. As DRV is extensively metabolized by cytochrome P450 3A4 and being a substrate of P-glycoprotein as well, it is co-administered with ritonavir (RTV) 100mg or cobicistat 150mg to increase its exposure [1,3-5]. Furthermore, concomitant food intake is advised to improve DRV bioavailability [6].

DRV is considered a safe and efficacious drug, however substantial pharmacokinetic variability has been reported [1,7]. Factors such as demographics, treatment adherence, concomitant medication and polymorphisms of cytochrome P450 3A4 iso-enzymes contributed to the observed variability [1,5,7]. Pharmacokinetic variability can potentially result in suboptimal DRV plasma concentrations. Suboptimal DRV plasma concentrations are highly undesirable as they can lead to drug resistance, insufficient virologic response or a virologic breakthrough in patients who earlier had an undetectable viral load [8]. Therapeutic drug monitoring (TDM) is therefore routinely performed to assure adequate drug exposure [8].

Despite the relatively long use and experience with DRV in daily practice, little is documented concerning the potential risk factors for a relatively low DRV concentration in HIV-infected individuals in an outpatient setting. The aim of the present study was to investigate the frequency as well as predisposing factors associated with DRV plasma concentration below the median population pharmacokinetic curve in an outpatient setting.

Methods

Study design and participants

We performed a retrospective study on demographics, measured DRV plasma concentrations and patient characteristics in HIV patients treated with DRV in the University Medical Center Groningen (UMCG), The Netherlands. All patients aged ≥18 years and using DRV were eligible for inclusion in this study. A database was created by extracting all measured DRV plasma concentrations from January 2010 to December 2014 from the UMCG electronic patient database. Due to intra-patient variability and potential dose-adjustments we considered only the first DRV level of each patient within 8 weeks after initiation of the DRV therapy for inclusion. DRV plasma concentrations were excluded if the time of ingestion relative to the collection of the blood sample was unknown. The ethical review board of the UMCG

evaluated the study protocol and waived the need for written informed consent due to the retrospective nature of the study (METc 2015.010).

Data collection and management

The following data were extracted from the medical records of the participants and included in the research database: sex, age, weight (during visit of drug level measurement), height, drug abuse, documented adherence, co-morbidity, serum creatinine concentration, AST, ALT, CD4+ cell count and viral load. The medical records of all participants were studied for medication potentially influencing the DRV concentrations.

When the possibility of non-adherence was suggested in the medical record, the patient was classified as 'potentially non-adherent'. Data concerning drug abuse included alcohol abuse (persistent use of ≥14 units of alcohol weekly) [9] and the use of recreational drugs. Co-morbidities with a known effect on pharmacokinetics were included in the database (e.g. renal-, hepatic-, and/or gastro-intestinal morbidity). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) formula [10]. The laboratory results (i.e. creatinine, AST, ALT, CD4+ cell count and viral load) included in the database corresponded with the date of the DRV plasma concentration or within a period of ±3 months. In order to interpret the hepatic function, the De Ritis ratio [11] was calculated and used.

Bioanalytical procedure and pharmacokinetic analyses

The concentrations of DRV in human plasma were analysed in the Laboratory of the Department of Clinical Pharmacy and Pharmacology at the UMCG by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS). All analyses were performed on a FinniganTM TSQ® Quantum Discovery (San Jose, USA) triple quadrupole LC–MS/MS with a FinniganTM Surveyor® LC pump and a FinniganTM Surveyor® autosampler. The calibration curves were linear within the concentration range of 0.335 to 33.5 mg/L for DRV and had a correlation coefficient (R^2) of 0.999. The lower limit of quantification (LLOQ) for DRV was 0.27 mg/L. This method was precise and accurate as within day precision ranged between 2.2% and 3.2% for DRV, and between-day precision ranged from 3.0% to 5.2%. The calculated accuracy ranged from 0.0% to 11.8%. Performance of the assay was within accepted limits of acceptance (accuracy and precision <10%) in our laboratory as confirmed by participation in the international proficiency testing program [12].

To estimate time-adjusted DRV plasma concentrations a DRV iterative two-stage Bayesian population pharmacokinetic model with the software package MWPharm 3.82 (Mediware, Groningen, The Netherlands) was used [13]. The model for DRV was a one-compartment model with input and elimination from the central compartment. Parameters for this model are: a volume of distribution of the central compartment of 2 L/kg (s.d. 0.5 L/kg), a total

body clearance of 6.3 L/h/1.85m^2 (s.d. 1.57 L/h/1.85m^2), first order absorption constant of 1/h (s.d. 0.25/h) and a bioavailability of 0.8 (in combination with RTV). This model was in-house built and derived from data provided in the literature [14]. Similar to standard care each time-adjusted DRV plasma concentration was compared with a median population pharmacokinetic curve for the once-daily or twice-daily dosage of DRV [15]. Consistent with daily practice the DRV plasma concentrations were dichotomized as either 'above' or 'below' the median curve. The median curves do not represent the minimal effective concentration, but are used in standard care as cut-off values for follow-up [15]. A DRV trough concentration below 1.07 mg/L for the once-daily dosage and 2.60 mg/L for the twice daily dosage is an indication for follow-up. This follow-up consists of repeated plasma drug concentration measurement, additional food intake advice and additional questions and guidance concerning therapy adherence.

Statistical analysis

Age, BMI, eGFR, De Ritis ratio, co-morbidity, drug abuse and documented adherence were tested for association with DRV plasma concentration above or below median population pharmacokinetic curve. We conducted a dichotomal logistic regression analysis with manual backward selection based on p-values to identify which set of variables best predicted a DRV plasma concentration below the median population pharmacokinetic curve. Co-morbidity, recreational drug abuse and documented adherence were marked as categorical covariates. We assessed the variance of the model using the Nagelkerke R square and we determined the goodness-of-fit by Hosmer-Lemeshow. Variables were checked for linearity and included as dummy variables (indicator variables) if necessary. All statistical analyses were performed using SPSS for Windows, version 22.0 (IBM SPSS, Chicago, Illinois).

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [16], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [17].

Results

In total 113 initial DRV plasma concentrations from 113 patients were measured within 8 weeks after initiation of DRV therapy and were therefore included in the research database. Ninety-four participants were using DRV/RTV 800/100mg once-daily and 19 participants were using DRV/RTV 600/100mg twice-daily. All participants were using RTV as a booster. Eighty-eight (78%) participants were among others using tenofovir simultaneously with DRV and 7 (6%) participants were using raltegravir simultaneously with DRV. No other

concomitant use of non-antiretroviral medication interacting with DRV was found after assessing the medical records of the participants. Further demographic characteristics are presented in table 1.

Table 1. Baseline demographic characteristics of the 113 study participants.

Characteristics	Value
Number of participants	113 (89 male)
Mean age (years)	43 (20 – 67)
Median BMI (kg/m2, IQR)	23.50 (21.1 – 26.0)
BMI subgroups (n(%))	
< 18 kg/m2	5 (4.4%)
18 – 25 kg/m2	64 (56.6%)
> 25 kg/m2	34 (30%)
Missing	10 (9%)
eGFR (mL/min/1.73 m2, mean (SD))	103.3 (23.3)
Median AST (IQR)	29 (23-35)
Median ALT (IQR)	25 (17.5-33.5)
Median CD4+ cell count (IQR)	430 (285-605)
Undetectable viral load in 55 patients	
Median Viral load in patients with detectable load (n = 58) (copies/mL, IQR)	600 (242 – 1486)
Documented co-morbidity	
Number of patients with hepatic morbidity	6
Number of patients with gastro-intestinal morbidity	4
Number of patients with renal morbidity	2
Drug abuse	
Recreational drugs	18
Alcohol	7
Documented potentially non-adherent	9

[1]BMI (body mass index) of these individuals could not be calculated as only body weight was recorded. IQR= interquartile range. SD= standard deviation, eGFR= estimated glomerular filtration rate, AST= aspartate aminotransferase, ALT=alanine aminotransferase.

The median (IQR) DRV plasma concentration was 2.8 (1.8 – 4.2) mg/L and 31 (27.4%) DRV plasma concentrations were classified 'below the median population pharmacokinetic curve'. In table 2 the patient characteristics and DRV drug concentrations below or above median population pharmacokinetic curve values are shown. Due to a lack of linearity with the dependant variable the variables age, eGFR and BMI were included in the logistic regression as dummy variables divided into 4 quartiles. The model best predicting a DRV level below the median population pharmacokinetic curve included the variables age and eGFR (Hosmer and Lemeshow Goodness of Fit Test p=0.945, Nagelkerke R Square 0.284). The results of the dichotomal logistic regression are presented in table 3.

Table 2. Patient characteristics and darunavir plasma concentrations below or above median population pharmacokinetic curve values.

Variable	Below population median (n = 31)	Above population median (n = 82)	P-value
Age (mean, years)	41	44	P= 0.138 (t-test)
Sex (male, %)	21 (68%)	68 (83%)	P = 0.078 (pearson chi-square)
BMI (median, IQR)	24.3 (21.3 – 27.5)	23.2 (20.8-25.7)	P=0.298 (MWU1)
eGFR, mean (SD)	114.5 (16.9)	99.0 (24.1)	P< 0.001, t-test
Earlier documented non-adherence (n(%))	5 (16.1%)	4 (4.9%)	P=0.062 (Fisher's exact)
Detectable HIV viral load (n(%))	17 (54.8%)	41 (50%)	P = 0.65 (pearson chi-square)
Recreational drugs /alcohol abusus	8 (26.8%)	17 (20.7%)	P= 0.56 (pearson chi-square)
De Ritis (median, IQR)	1.3 (0.9-1.6)	1.1 (0.9-1.5)	P= 0.33 (MWU)

[1]MWU = Mann-Whitney U test

Table 3. Results of the dichotomous logistic regression analysis with manual backward selection.

			95% CI for odds ratio	
Variable	**Significance**	**Odds ratio**	**Lower**	**Upper**
Age				
35 years	0.025			
43 years	0.028	3.916	1.161	13.207
53 years	0.021	5.608	1.298	24.240
>53 years	0.712	0.634	0.056	7.138
eGFR				
89 mL/min	0.096			
106 mL/min	0.070	7.744	0.845	70.969
116.5 mL/min	0.020	15.246	1.533	151.579
>116.5 mL/min	0.016	19.458	1.721	220.025

Discussion

In this descriptive study we assessed the predictive value of patient- and disease- related factors on DRV plasma concentrations after initiation of the DRV therapy. In a dichotomous logistic regression analysis we found that the combination of age and eGFR were associated with a DRV plasma concentration below the median population pharmacokinetic curve.

Like other antiretroviral PIs, DRV pharmacokinetics are characterized by large inter-patient variability [1,7]. The observed variability could partly be caused by inadequate food intake as DRV is advised to be taken concomitantly with food to achieve adequate exposure [6]. In order

to identify other potential contributors to a DRV plasma concentration below the median population pharmacokinetic curve, besides food intake, in this study we focused on several patient- and –disease- related factors. More than a quarter (27.4%) of the DRV plasma concentrations were below the median curve triggering physicians to change their follow-up strategy. Theoretically it would be expected that 50% of the DRV plasma concentrations would be below the median curve. The used median population pharmacokinetic curves do not have a relation with therapeutic effectiveness of DRV, but are used as a cut-off value in standard care to determine whether follow-up is prudent [15,18]. Follow-up consists of an additional food intake advice, additional questions and guidance concerning therapy adherence and a repeated blood sample for DRV monitoring. Therefore in daily practice physicians are aiming for higher then median DRV plasma concentrations.

That eGFR is found to be a predictor for below median DRV plasma concentration in combination with age in the current study is a remarkable finding, since DRV is mainly eliminated by the liver. A higher than average eGFR alone would therefore presumably have a minor impact on DRV plasma concentration in daily practice. The observed inverse relation between eGFR and DRV concentrations in the current study might potentially be induced by the concomitant use of tenofovir. During the study period 78% of the participants were using tenofovir simultaneously with DRV. A higher risk of renal impairment has been reported in patients receiving tenofovir and a RTV boosted PI, such as DRV [19]. In addition, the summary of product characteristics (SPC) of DRV demonstrates that co-administration of tenofovir (300 mg once daily) and DRV/RTV (300/100 mg bid) increased DRV AUC, C_{max} and C_{min} by 21%, 16% and 24%, respectively [20]. The observed inverse relation between eGFR and DRV concentration might be due to concomitant tenofovir usage, which is nephrotoxic on the one hand and inhibits DRV clearance on the other [19,20]. Earlier studies suggested that raltegravir also could lower DRV concentrations [21,22]. Of the 7 participants using raltegravir and DRV simultaneously in the current study, 2 had a DRV concentration below median curve. Unfortunately due to the unequally divided and small sample sizes of both tenofovir and raltegravir users and non-users a subanalysis was not possible.

The other variable in the prediction model was age. The highest quartile of the variable age (>53 years old) showed lower risk of a DRV plasma concentration below median curve in combination with eGFR. The effect of age on plasma antiretroviral drug (PI) exposure was shown earlier 23. The plasma concentrations of DRV might be higher with increasing age due to a lowered hepatic (CYP450 3A4 enzyme) activity in older people [24,25].

An important limitation in the current study is that no data concerning concomitant food intake with DRV ingestion was available. The observed variability in DRV concentrations in the current study might potentially be confounded by inadequate concomitant food intake, although that is unlikely based on a prior study assessing food intake concomitantly with

DRV [26]. Another weakness is the limited number of events in the model. Nevertheless, the results of this study provide an insight into the DRV plasma concentrations after start of therapy at an HIV outpatient clinic.

Linking several patient- and disease-related factors to the routinely measured DRV plasma concentrations shows that younger patients with a higher than average eGFR more frequently have a DRV plasma concentration below median curve. The impact of tenofovir has be to clarified in future studies in populations with a larger proportion of tenofovir-free regimens to facilitate interpretation. Although TDM is not a substitute for clinical judgement it can be a powerful tool identifying patients with lower DRV concentrations and subsequently at risk for drug resistance.

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