

# Darunavir Concentrations in Cerebrospinal Fluid and Blood in HIV-1–Infected Individuals

Aylin Yilmaz,<sup>1</sup> Arash Izadkhashti,<sup>1</sup> Richard W. Price,<sup>2</sup> Patrick W. Mallon,<sup>3</sup>  
Marc De Meulder,<sup>4</sup> Philip Timmerman,<sup>4</sup> and Magnus Gisslén<sup>1</sup>

## Abstract

Darunavir is the most recently licensed protease inhibitor currently used in treatment-experienced HIV-infected individuals. Our objective was to determine darunavir concentrations in cerebrospinal fluid (CSF) and plasma in subjects receiving antiretroviral treatment regimens containing ritonavir-boosted darunavir. Darunavir concentrations were determined by liquid chromatography tandem mass spectrometry in 14 paired CSF and plasma samples from eight HIV-1-infected individuals. The lower limit of quantification was 5.0 ng/ml. All of the 14 CSF samples had detectable darunavir concentrations with a median darunavir concentration of 34.2 ng/ml (range 15.9–212.0 ng/ml). The median (range) plasma darunavir concentration was 3930 (1800–12900) ng/ml. All CSF samples had detectable darunavir concentrations. Most of them exceeded or were in the same range as levels needed to inhibit replication of wild type virus, making it probable that darunavir, at least to some extent, contributes to the suppression of HIV replication in the central nervous system.

## Introduction

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) has considerably reduced the incidence of HIV-associated morbidity and mortality. Antiretroviral treatment has also had a tremendous impact on the occurrence of neurological complications, including not only central nervous system (CNS) opportunistic infections but also the AIDS dementia complex related to more direct infection of the CNS.<sup>1</sup> This is most probably attributable to improvements in immune function alongside a reduction of the viral burden and inflammatory processes in the CNS resulting from effective treatment.

For an antiretroviral drug to effectively inhibit viral replication in the CNS, it must be able to penetrate the blood–brain barrier (BBB). The capacity of a drug to enter the CNS depends on a number of factors: molecular size, lipophilicity, degree of ionization and plasma protein binding, and whether or not the drug is a substrate for transmembrane transporters.

The protease inhibitors (PIs) are in general large molecules highly bound to plasma proteins such as  $\alpha_1$ -acidic glycoprotein (AAG) and albumin. In addition, they are to varying extent substrates for P-glycoprotein, a transport pump located on brain endothelial cells, making PIs more or less susceptible to active efflux from the CNS.<sup>2</sup> All these characteristics make it more difficult for PIs to traverse the BBB, and as a conse-

quence, CSF concentrations of most PIs are low or undetectable.<sup>3–6</sup> However, as PIs are very potent drugs, only small concentrations may be required to inhibit viral replication within a sanctuary site such as the CNS.

Darunavir is the most recently licensed PI. The coadministration of darunavir with low-dose ritonavir leads to a several-fold increase of the systemic exposure to darunavir.<sup>7</sup> It has high *in vitro* activity against many viral strains resistant to other PIs,<sup>8</sup> and has clinically been shown to be effective in both treatment-naïve as well as heavily treatment-experienced subjects.<sup>9–11</sup> It is currently licensed for use in treatment-experienced patients. Like most other PIs, it is highly bound to plasma proteins (95%), with a small fraction (5%) of unbound drug available to penetrate the CNS. The objective of this study was to determine if darunavir, when administered with low-dose ritonavir (darunavir/r), could traverse the BBB and achieve sufficient concentrations in CSF.

## Materials And Methods

### Patients

A total of eight HIV-1-infected subjects were included from Gothenburg, Sweden ( $n = 5$ ), San Francisco, CA ( $n = 2$ ), and Dublin, Ireland ( $n = 1$ ). Patient characteristics are presented in Table 1. All patients were on HAART containing

<sup>1</sup>Department of Infectious Diseases, the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

<sup>2</sup>Department of Neurology, University of California San Francisco, San Francisco General Hospital, San Francisco, California 94110.

<sup>3</sup>University College Dublin School of Medicine and Medical Sciences, Mater Misericordiae University Hospital, Dublin, Ireland.

<sup>4</sup>Johanson & Johnson Pharmaceutical Research and Development, Beerse, Belgium.

TABLE 1. PATIENT CHARACTERISTICS INCLUDING PLASMA AND CSF DARUNAVIR CONCENTRATIONS FOR EACH SUBJECT<sup>a</sup>

Patient number	Visit number	Sex	Age	CD4 cell count ( $\times 10^6$ /liter)	Plasma VL ( $\log_{10}$ copies/ml)	CSF VL ( $\log_{10}$ copies/ml)	Antiretroviral treatment in addition to drv/r	Time on ART (weeks)	Drv concentrations (ng/ml)	
									Plasma	CSF
1	1	m	57	320	2.51	<1.30	zdv + 3TC + abc + T20 + ral	13.0	2680	34.9
	2	m	57	110	<1.30	<1.30	zdv + 3TC + abc + ral	43.5	8550	96.5
2	1	m	46	160	<1.30	<1.30	zdv + 3TC + abc + ral	16.0	3870	25.6
	2	m	46	190	<1.30	<1.30	zdv + 3TC + abc + ral	66.0	1800	19.6
3	1	m	61	430	3.73	<1.30	zdv + 3TC + abc + T20	12.0	3210	33.5
	2	m	61	370	1.82	<1.30	zdv + 3TC + abc + T20	50.0	6740	53.8
4	1	m	43	80	<1.30	<1.30	zdv + 3TC + abc + ral	20.5	4060	35.5
	2	m	43	140	<1.30	<1.30	zdv + 3TC + abc + ral	56.0	3990	23.5
5	1	f	22	0	5.20	5.66	zdv + 3TC + abc + ral	0.5	12900	212.0
6	1	m	49	38	4.05	<1.30	zdv + 3TC + tdf + ral	0.9	9120 <sup>b</sup>	43.9 <sup>b</sup>
	2	m	49	92	2.66	<1.30	zdv + 3TC + tdf + ral	4.0	3770	28.7
7	1	m	50	133	4.10	4.00	tdf + ftc + ral	1.0	4590	15.9
	2	m	50	140	2.35	1.32	tdf + ftc + ral	4.9	2820	20.1
8	1	m	54	168	2.38	<1.30	zdv + 3TC + abc	2.5	3550	64.8

<sup>a</sup>VL, viral load; ART, antiretroviral treatment; m, male; f, female; zdv, zidovudine; 3TC, lamivudine; abc, abacavir; drv/r, darunavir/ritonavir; T20, enfuvirtide; ral, raltegravir; tdf, tenofovir; ftc, emtricitabine.

<sup>b</sup>Sampling time unknown.

darunavir/ritonavir 600/100 mg twice daily. Two of the patients had neurological complications; one was diagnosed with HIV-encephalitis and the other with atypical mycobacterial infection involving the CNS. The other six were neurologically asymptomatic and underwent a lumbar puncture in the context of longitudinal cohort studies.<sup>12</sup> Seven of the patients were male. The median (range) age was 50 (22–61) years. The CD4<sup>+</sup> T cell counts ranged from 0 to 430  $\times 10^6$  cells/liter, with a median value of 140  $\times 10^6$  cells/liter.

This study was approved by the ethics committees of the University's of each center.

### Methods

Lumbar punctures were conducted in a standardized manner, and CSF and plasma specimens were immediately stored at  $-70^\circ\text{C}$  until analysis. CSF levels of darunavir were determined in the laboratories of Johnson and Johnson Pharmaceutical Research and Development, Beerse, Belgium. Total (i.e., bound + unbound) plasma and CSF levels of darunavir were determined using a liquid chromatography tandem mass spectrometry (LC-MS/MS) method. After matrix matching with 50- $\mu\text{l}$  aliquots of blank human CSF or plasma, respectively, the 14 paired plasma and CSF samples from the eight patients (50- $\mu\text{l}$  aliquots) were spiked with 50  $\mu\text{l}$  of internal standard solution in acetonitrile. Calibration curves (Cal) and independent quality control samples (QC) (at three concentration levels in duplicate) were prepared in the same matrix as the study samples. These Cal and QC samples were analyzed together with the study samples. Precipitation was done by the addition of 0.5 ml acetonitrile. The samples were centrifuged and 10  $\mu\text{l}$  of the supernatant was injected onto a reversed-phase column (3 cm  $\times$  4.6 mm, packed with 3.5  $\mu\text{m}$  Xbridge C<sub>18</sub>, Waters) for final separation and quantification. LC-MS/MS analysis was carried out on an API-4000 triple quadrupole mass spectrometer (Applied Biosystems), which was coupled to a high-performance liquid chromatography

pump (Agilent) and autosampler (Shimadzu). The quantification limit was 5.0 ng/mL. Linearity of the Cal ranged from 5.0 to 10,000.0 ng/ml. Acceptance criteria of the bioanalytical run were in line with current Food and Drug Administration (FDA) guidance on bioanalysis with respect to accuracy and precision on Cal and QC samples.

HIV-1 RNA was quantified in CSF and plasma with COBAS Amplicor HIV-1 Monitor Test, version 1.5 (Roche AB, Basel, Switzerland) and run according to the manufacturer's protocol. The assay has a dynamic range down to 50 copies/ml (1.70  $\log_{10}$  copies/ml) and a lower detection limit of 20 copies/ml (1.30  $\log_{10}$  copies/ml). All HIV-1 RNA values <20 copies/ml were estimated to 19 copies/ml (1.28  $\log_{10}$  copies/ml). The peripheral blood CD4<sup>+</sup> T cell count was measured by direct immunofluorescence in a flow cytometer.

### Statistical analysis

Nonparametric methods were used for group descriptives and for darunavir concentrations (median and range).

### Results

Total CSF and plasma darunavir concentrations are presented in Fig. 1 and Table 1. At the time of sampling patients had been receiving darunavir for a median (range) of 12.5 (0.5–66.0) weeks. All 14 CSF samples had detectable darunavir concentrations ranging from 15.9 to 212.0 ng/ml (median 34.2 ng/ml). The median (range) plasma darunavir concentration was 3930 (1800–12900) ng/ml. The CSF-plasma ratios ranged from 0.3 to 1.8% (median 0.9%). Darunavir concentrations in all 14 CSF samples were in the range of or above the protein-adjusted IC<sub>50</sub> (drug concentration needed to inhibit 50% of viral replication) of 12–55 ng/ml.<sup>8</sup> Eleven out of 14 CSF samples had HIV RNA levels <1.28  $\log_{10}$  copies/ml, and all patients except subject number 5, with atypical mycobacterial infection in the CNS, had higher viral loads in plasma than in CSF. Among the patients with detectable CSF

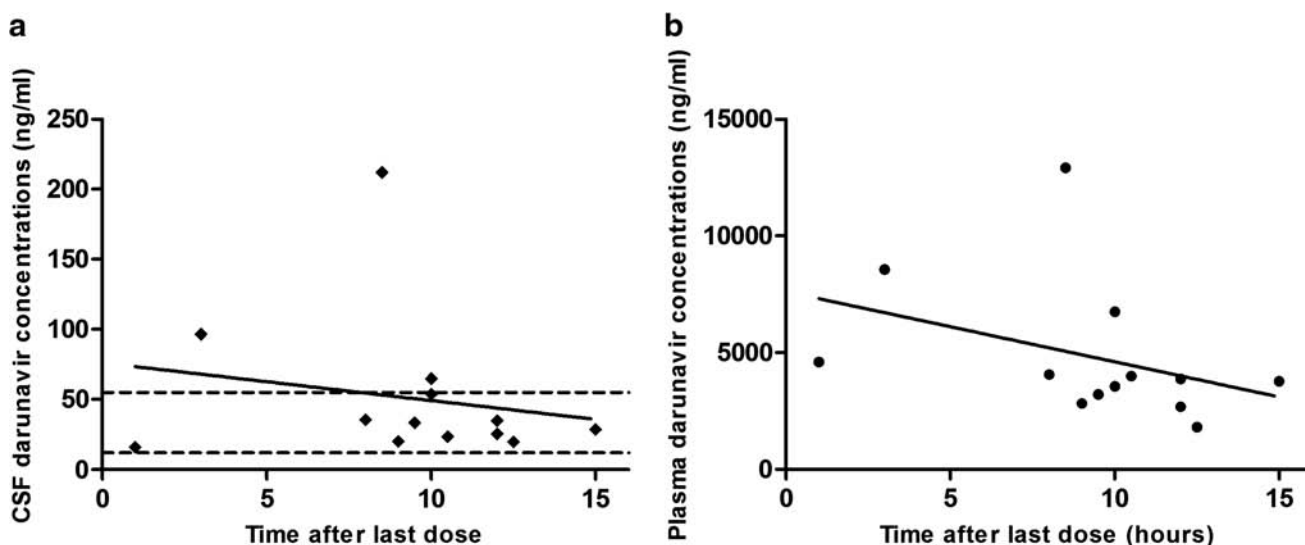


FIG. 1. Darunavir cerebrospinal fluid (CSF) (a) and plasma (b) concentrations by postdose time for the 13 paired specimens with known sampling time. The solid lines are linear regression lines for CSF and plasma. The dotted lines in (a) indicate the estimated range of the IC<sub>50</sub> in CSF (12–55 ng/ml).

viral loads, two had been on treatment for only 1 week or less. The third subject had received antiretroviral therapy with tenofovir, emtricitabine, and raltegravir in addition to darunavir/ritonavir for almost 5 weeks.

## Discussion

The PIs have a number of unfavorable characteristics, which make it more difficult for them to traverse the BBB. Most of them, including darunavir, are highly bound to plasma proteins. Darunavir was detected in all 14 CSF samples, but the drug concentrations in CSF were only around 0.9% of plasma concentrations. This could be due to active transport out of CSF or limited entry into CSF.

The IC<sub>50</sub> value for darunavir of 12–55 ng/ml used in this study was obtained *in vitro* from acutely infected MT-4 cells, peripheral blood mononuclear cells, and in macrophages in the presence of 50% human serum.<sup>8</sup> The addition of human serum leads to a substantial increase of the IC<sub>50</sub> for most PIs, for darunavir about 20-fold. The CSF contains far less proteins than plasma, but we do not know the degree of drug binding to proteins in CSF, and therefore it is difficult to determine an IC<sub>50</sub> adjusted for this compartment. Probably, the IC<sub>50</sub> for CSF is somewhere between the IC<sub>50</sub> without and with 50% human serum. All CSF samples had darunavir levels exceeding 12 ng/ml, suggesting that darunavir, at least to some extent, might contribute to the inhibition of wild-type HIV replication in the CNS. It should, however, be kept in mind that drug concentrations in CSF do not directly correlate with drug levels reached within the brain parenchyma or to antiviral effect. In contrast, lopinavir and indinavir are PIs that have previously been shown to penetrate into the CNS with CSF levels exceeding the IC<sub>50</sub> several-fold.<sup>13–15</sup> Saquinavir/ritonavir, nelfinavir, and atazanavir lead to undetectable or very low CSF concentrations, and do not fully suppress viral replication in CSF when used as monotherapy.<sup>6,16,17</sup>

The highest CSF darunavir concentration was observed in patient number 5 who had an atypical mycobacterial infection

involving the CNS at the time of sampling. An ongoing infection in the CNS compromises the BBB and might theoretically lead to increased drug distribution into the CNS and higher CSF drug concentrations. This patient, however, also had the highest darunavir level in plasma, which is probably the explanation for the high darunavir level in the CSF. The second patient with neurological complications, patient number 8 with HIV-encephalitis, had a CSF darunavir concentration of 64.8 ng/ml.

We cannot draw far-reaching conclusions about the efficacy, or lack of efficacy, of darunavir/r in CNS from this study because all patients were on concomitant treatment with at least three other antiretroviral drugs. Subject number 7 had detectable CSF HIV RNA despite almost 5 weeks on treatment. There are no published human data on the CNS distribution for two of the drugs in his regimen (raltegravir and emtricitabine), and tenofovir reaches only low concentrations in the CSF.<sup>18</sup> The majority of the patients with suppressed CSF viral loads were on triple-NRTI treatment with zidovudine, abacavir, and lamivudine; these drugs are known to penetrate the BBB.<sup>19–21</sup> In addition, two of the patients (numbers 5 and 6) had received treatment for less than 1 week, making it somewhat uncertain whether they had reached steady-state concentrations of darunavir in the CSF.

The effectiveness of antiretroviral drugs and drug combinations in the CNS has been a subject of interest for almost two decades. Results thus far suggest that most combinations previously used are also effective in the CNS,<sup>13,22,23</sup> although some antiretroviral drugs, such as atazanavir, saquinavir, and nelfinavir, do not treat HIV inside the CNS as well as in the periphery.<sup>6,16,17</sup> Most HIV-infected individuals initiating HAART will have a marked reduction of their CSF viral load,<sup>24–26</sup> and a larger number of them will reach HIV RNA levels <2.5 copies/ml in CSF than in plasma.<sup>27,28</sup> This could probably, at least in part, be explained by the fact that most subjects have lower pretreatment viral loads in CSF than in plasma. Differences in the predominant infected cell types in each compartment might also contribute.<sup>29–31</sup> In contrast, CSF

levels of neopterin, a marker of the cell-mediated immune activation, will remain elevated in a large number of patients despite many years of suppressive therapy.<sup>32</sup> We do not know the long-term consequences, if any, of having an activated immune system inside the CNS for a prolonged time. Our previous findings suggest that this persisting intrathecal immune activation observed in treated patients is mainly driven by residual viral replication within the CNS. By suppressing the viral replication in the CNS to a maximum, it should be possible to further diminish the immune activation in the CNS.<sup>33</sup> If this turns out to be the case it would seem even more important to start patients on regimens with a high degree of CNS penetration.

Virological failure and drug resistance are problems seen in some of HAART-treated individuals. Increased viral replication and immune activation may also take place inside the CNS, rendering such patients at increased risk for neurological complications and neurocognitive deficits. In this category of patients it could be important to consider the neuroeffectiveness of the antiretroviral drugs when putting together a regimen. This issue might become significant in patients receiving novel or not commonly used antiretroviral drug combinations, such as NRTI-sparing regimens. The use of darunavir as part of HAART is increasing around many parts of the world, mainly in experienced patients. All patients in this study had detectable levels of darunavir in CSF with concentrations in the range of, or exceeding the IC<sub>50</sub> for wild-type virus making it probable that darunavir, at least to some extent, contributes to the suppression of HIV replication in the CNS.

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### Disclosure Statement

No competing financial interests exist.

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Address reprint requests to:

Aylin Yilmaz

Department of Infectious Diseases  
Sahlgrenska University Hospital/Östra  
Smörslottsgatan 1  
416 85 Göteborg, Sweden

E-mail: aylin.yilmaz@infect.gu.se

