**Doravirine Achieves High CSF Exposure and is Mostly Unbound to Proteins**

 (Spanish HIV/AIDS ResearchNetwork, PreEC/RIS65)

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**Abstract**
We determined total and unbound concentrations of Doravirine (DOR) in cerebrospinal fluid (CSF) in 15 asymptomatic, HIV virologically suppressed patients. The median (range) plasma and CSF total DOR concentrations were 417.6 ng/mL (169.5–942.2) and 58,6 ng/mL (23.2–127.3), respectively. Median (range) plasma and CSF unbound DOR concentration were 53.5 ng/mL (21.3–115.5) and 44.6 ng/mL (14.9-105.0). Total and unbound DOR concentrations in CSF exceeded the EC50 value against wild-type virus (5.1 ng/mL) in all subjects suggesting that DOR may contribute to inhibit viral replication in this compartment.

**Keywords**
Doravirine; CNS; CSF; concentrations; unbound; HIV.

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**Background**

Current antiretroviral therapy (cART) has demonstrated to be highly effective and safe for the treatment of people living with human immunodeficiency virus and/or acquired immunodeficiency syndrome (PLWHA). Available cART combinations maintain HIV viral load suppressed in blood plasma in almost all situations. This has contributed to reduce morbidity and mortality rates as well as improve people's quality of life. However, even in well treated patients, a limited diffusion of some antiretrovirals through the natural barriers (such as the blood-brain barrier) may allow HIV to persist and replicate within these compartments[1].

Doravirine (DOR) is a new HIV-1 non-nucleoside reverse transcriptase inhibitor (NNRTI) that has demonstrated a good efficacy and safety profile in clinical trials [2]. DOR inhibits viral replication of both wild-type virus and most common NNRTI variants. Initial pharmacokinetic studies demonstrated a time to maximal concentration of 1–5 h, not extensive binding to plasma proteins (76%) and poor solubility in water. In animal models, the tissue distribution was assessed using 14C-labelled DOR. Low levels of radioactivity were observed in the brain suggesting that DOR does not readily cross the blood-brain barrier[3].

In this study, we sought to evaluate the pharmacokinetics of DOR in cerebrospinal fluid (CSF) of HIV-infected individuals and its relationship with CSF viral suppression.

**Methods**

This is a single-arm, open-label, single-centre study. 15 asymptomatic HIV-infected virologically suppressed adult patients were selected and enrolled from our HIV outpatient clinic. All participants switched from stable ART to DOR plus Emtricitabine (FTC)/ Tenofovir Alafenamide (TAF). After 4 weeks, total and unbound DOR concentrations in blood plasma, as well as CSF, were measured 24 hours postdose. Total DOR concentrations in plasma were determined using a liquid-liquid extraction (TBME) method, with a calibration range intaiintai2-2.000 ng/mL (linear 1/x2) in drug-free plasma, while total DOR concentrations in CSF were determined using a protein precipitation (acetonitrile) method, with a calibration range 0.2-200 ng/mL (linear 1/x2) in artificial CSF. 13C6 DOR was used as an internal standard. HIV-1 RNA was quantified in plasma and CSF by real-time polymerase chain reaction technique (RT-PCR; Abbot Molecular, Des Plaines, IL) performed according to the manufacturer’s recommendations (lower limit of quantification, 20 copies/mL). The study was approved by the hospital ethics committee and the Spanish Agency of Medicines and medical devices, and patients gave written informed consent to participate (EudraCT Number: 2018-003915-24)

**Results**

A total of 15 plasma and 15 CSF samples were collected. Most subjects were male (86%), with a median age of 46 years (min-max 27-66) and body mass index (BMI) of 24.1 Kg/m2 (min-max 18.5-33.8). The median nadir CD4 count was 315 cell/mL (min-max 24-513), median RNA HIV viral load (VL) at the moment of HIV diagnosis, was 28.923 copies/mL (min-max 2.340-10.681.306). Only 5 participants (33%) had a history of AIDS-stage disease (CDC Classification). Current median CD4 count was 704 cell/mL (min-max 365-1744). All patients were in stable ART for at least 6 months [median 108 months (min-max 23-268)]. HIV had been suppressed (RNA HIV-1 < 20 copies/mL) during the last 3 months, in all participants. Most patients switched from Integrase Strand Transfer Inhibitor (INSTI)-based regimens (80%).

Median total and unbound DOR concentrations in blood plasma were 417.6 ng/ml (min-max 169.5-942.2) and 53.5 ng/ml (min-max 21.3-115.5) respectively (free plasma fraction 12,7%); while in CSF were 58.6 ng/ml (min-max 23.2-127.3) and 44.6 ng/ml (min-max 14.9-105) (free CSF fraction 75.9%)(Figures 1 a&b). Total DOR CSF concentrations to total plasma ratio was 0.12 and DOR total CSF to unbound plasma ratio was 0.99. Only one non-adherent patient presented with detectable viral load in blood plasma (76 copies/mL), undetectable viral load was confirmed in a further control. On the other hand, one subject presented with asymptomatic low-level viral replication in CSF (32 copies/ml) while undetectable in plasma [Table 1]. Median CSF cell count, glucose, and proteins were 0.001 × 109/L (min-max 0.001-0.159), 3.7 mmol/L (min-max 2.8–6.8), and 0.29 g/L (min-max 0.14–0.68), respectively. Median CSF/plasma albumin ratio was 3.96 (range, 1.95-9.17) suggesting no increased blood-brain barrier permeability.

Of note, 2 patients wrongly maintained their previous treatment (BIC/TAF/FTC) while receiving DOR. No adverse events or side effects were reported. Total and unbound DOR concentrations in plasma and CSF remained similar to the rest of participants. We also measured BIC concentrations in these patients (week 4 visit) and we found adequate conentrations which suggest no significant drug-drug (DOR-BIC) interactions. In general, DOR + FTC/TAF was well tolerated and no significant AEs were reported.

**Discussion**

We conducted a pilot, cross-sectional study, looking at DOR plasma and CSF exposure and viral suppression. Eighty seven percent of DOR was bound to plasma proteins. Twelve percent of total plasma DOR was found in CSF, moreover 76% of it was protein-free. Total and unbound DOR concentrations in CSF exceeded the half-maximal effective concentration (EC50 5.1 ng/mL) value against wild type (WT) virus, maintaining viral suppression in this compartment in all but one participant.

Asymptomatic HIV replication may occur within the CNS in the absence of systemic HIV replication. In these cases HIV RNA can be detected in cerebrospinal fluid (CSF) but not in blood. This discordance between the CSF and the blood is known as “CSF viral escape”and it has been extensively reported in the literature. In a large cohort study published recently, authors reported CSF viral escape as prevalent as 4.4% [4]. We found no evidence of suboptimal antiretroviral levels in this case. Moreover this patient was concomitantly receiving DOR plus BIC/FTC/TAF, which suggests that viral escape may occur regardless of adecuate antiretroviral treatment.

Achieving HIV suppression in blood plasma seems to be the main goal of ART, however several studies suggest that the CNS may act as an independent compartment and severe complications such as HIV-encephalitis have been previously reported. Moreover asymptomatic low-level viral replication may result in persistent low level immflamation within the CNS and consequent neuronal damage and neurocognitive impairment [5]. Hence achieving therapeutic concentrations within this compartiment may help to prevent this complications.

ARV drugs penetrate into the CNS differently. In absence of conditions that alter the permeability of the blood brain barrier, drug concentrations are usually influenced by several factors, including molecular size, lipophilicity, plasma protein binding and active transport. Nevirapine (NVP) the first NNRTI to be licenced (still widely used worldwide), is poorly bound to plasma proteins (60%) and its CSF exposure remains high (CSF to plasma ratio: 28-45%). Contrary, other NNRTIs such as Efavirenz (EFV), Etravirine (ETR) and Rilpivirine (RPV) do not readly cross the blood brain barrier (0,5%; 1% and 1.4% respectively), however they achieve sufficient concentrations to maintain viral suppression in this compartment when compared to EC50-90[6].

Contrary to what was described in the literature, we found that DOR was 87% bound to plasma proteins (previously reported 76%). Similar data was recently presented at an international conference [7]. These discrepancies may lay in methodological differences (e.g. in-vitro experiments using plasma spiked with [3H]doravirine and measured using liquid scintillation counting). To our knowledge, there are currently no data on the extent of doravirine binding in PLWH.

In our study we found that DOR concentration was 12% of that in blood plasma, and that the CSF concentration was almost similar to the unbound fraction in plasma (total CSF/unbound plasma ratio was 0.9) which suggest that DOR crosses the blood-brain barrier by passive diffusion. Interestingly Darunavir (DRV), the most widely used protease inhibitor, is highly bound to plasma proteins but is predominantly unbound to protein in cerebrospinal fluid [8].

 INSTIs based regimens are currently the first choice in all international guidelines. Around 5% of Raltegravir concentrations in blood plasma are found in CSF. CSF concentrations exceed the IC95 and it used to be included in regimens designed for improved CSF penetration. Viral suppression as well as CSF exposure with second generation INSTI drugs have been recently studied by our group and others. Dolutegravir and Bictegravir reach effective concentrations in the CSF. However, unbound DTG and BIC concentrations in CSF are only 23% and 35% of total drug in CSF respectively [9-10].

The ratio of drug concentration achieved in the CSF to the known EC50-95 refers to the CSF inhibitory quotient (IQ). This a useful concept to estimate antiviral activity and can be used to compare different antiretroviral drugs activity within the CNS. Because only protein-free drug is active, when the CSF unbound fraction is known, a new approach, the *“freeCSF IQ”* (free[ ]CSF/EC50-95) may be more accurate. The known greater potency of BIC and DTG result in a high *freeCSF IQ*, BIC 2.2 and DTG 8.5. However as most of the CSF DOR is unbound we found that DOR *freeCSF IQ* is higher tan BIC and similar to DTG (8.6).

In conclusion DOR exposure is high as well as its protein-free concentrations in CSF. These results suggest that DOR, in combination with other ARV drugs with known antiviral activity within the CNS , should be used in those patients with HIV-associated neurologic complications.

**Notes**

**Disclaimer.** Merck Sharp & Dome (MSD) was given the opportunity to review a preliminary version of this article for factual accuracy. The authors are solely responsible for the study design, interpretation of results, and final content of the article.

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### Table 1: Patient’s Characteristics: CSF and Plasma Results

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PATIENT# | Gender/Age (years) | BMI | Nadir CD4 | Current CD4 | CSF HIV RNA copies/ml | Plasma HIV RNA copies/ml | Plasma DOR ng/ml | CSF DOR ng/ml | Unbound Plasma DOR ng/ml | Unbound CSF DOR ng/ml | DOR totalCSF/totalplasma ratio | DOR totalCSF/unboundplasma ratio |
| 1 | M/38 | 33,8 | N/A | 686 | <20 | <20 | 385,9 | 38,7 | 37,8 | 24,7 | 0,10 | 1,02 |
| 2 | M/60 | 25,6 | 24 | 1144 | <20 | <20 | 942,2 | 127,3 | 104,7 | 105,0 | 0,14 | 1,22 |
| 3 | M/46 | 23 | 192 | 636 | <20 | <20 | 281,7 | 37,2 | 36,0 | 25,8 | 0,13 | 1,03 |
| 4 | M/28 | 27,8 | 409 | 869 | <20 | <20 | 535,7 | 66,2 | 89,2 | 50,8 | 0,12 | 0,74 |
| 5 | M/55 | 27,2 | 432 | 640 | 32 | <20 | 546,4 | 79,4 | 61,2 | 60,3 | 0,15 | 1,30 |
| 6 | M/65 | 24,1 | 468 | 704 | <20 | <20 | 912,9 | 102,8 | 115,5 | 80,6 | 0,11 | 0,89 |
| 7 | M/66 | 24,4 | 83 | 667 | <20 | <20 | 447,1 | 40,9 | 60,5 | 29,0 | 0,09 | 0,68 |
| 8 | M/33 | 22,9 | 315 | 1077 | <20 | <20 | 313,9 | 38,0 | 39,7 | 26,8 | 0,12 | 0,96 |
| 9 | M/49 | 25,4 | 254 | 633 | <20 | <20 | 527,8 | 58,6 | 71,5 | 44,6 | 0,11 | 0,82 |
| 10 | M/27 | 18,5 | 513 | 937 | <20 | 76 | 169,5 | 26,1 | 21,3 | 17,9 | 0,15 | 1,22 |
| 11 | M/47 | 28,2 | N/A | 773 | <20 | <20 | 535,1 | 59,1 | 58,0 | 48,7 | 0,11 | 1,02 |
| 12 | M/31 | 22,7 | 233 | 372 | <20 | <20 | 181,6 | 23,2 | 24,7 | 14,9 | 0,13 | 0,94 |
| 13 | F/46 | 21,7 | 456 | 1744 | <20 | <20 | 230,3 | 28,8 | 36,7 | 17,8 | 0,12 | 0,78 |
| 14 | M/57 | 23,4 | 170 | 1296 | <20 | <20 |  N/D | 85,5 |  N/A | 72,1 | N/A | N/A |
| 15 | F/31 | 20,5 | 360 | 379 | <20 | <20 | 388,1 | 75,0 | 49,0 | 64,6 | 0,19 | 1,53 |
| Median(Min-Max) | 46(27-66) | 24,1(18,5-33,8) | 315(24-513) | 704(365-1744) | <20 | <20 | 417,6(169,5– 942,2) | 58,6(23,2 – 127,3) | 53,5 (21,3-115,5) | 44,6(14,9-105,0) | 0,12(0,09 – 0,19) | 0,99 (0,68-1,53) |

Abbreviations: BMI, body mass index; CSF, cerebrospinal fluid; DOR, Doravirine; HIV, human immunodeficiency virus; F, female; M, male; Min, minimum; Max, maximum; N/A, not applicable; RNA, ribonucleic acid

**Figure 1 a&b: Total and unbound DOR concentration in blood plasma and CSF.**

