Antiviral activity and CSF concentrations of 600/100 mg of darunavir/ritonavir once daily in HIV-1 patients with plasma viral suppression

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Objectives: The objective of this study was to assess whether a lower dose than the currently used one of darunavir/ritonavir might achieve good CSF concentrations and contribute to inhibition of CNS viral replication.

Patients and methods: This was a substudy of a randomized, open, multicentre study (eudraCT 2011-006272-39), comparing the efficacy and safety of 800/100 mg of darunavir/ritonavir (darunavir 800) versus 600/100 mg of darunavir/ritonavir (darunavir 600) once daily plus tenofovir/emtricitabine or abacavir/lamivudine in 100 virologically suppressed patients. Paired blood and CSF samples were obtained. Total plasma darunavir concentrations were determined by HPLC, and CSF concentrations by liquid chromatography–tandem MS. Viral load (VL) was determined in plasma and CSF (limit of detection = 40 copies/mL) by PCR.

Results: Sixteen patients were enrolled. The median (range) of darunavir CSF concentrations in darunavir 600 (n=8) and darunavir 800 (n=8) patients was 17.08 (5.79–30.19) and 13.23 (3.47–32.98) ng/mL, respectively (P=0.916). The median (range) darunavir CSF:plasma ratio was 0.010 (0.005–0.022) in darunavir 600 patients and 0.008 (0.004–0.017) in the darunavir 800 arm (P=0.370). All 16 patients had an VL<40 copies/mL in plasma and 14 had a VL<40 copies/mL in CSF. Of the two patients with detectable CSF VL (280 copies/mL and 159 copies/mL), one was receiving darunavir 600 and the other darunavir 800 plus tenofovir/emtricitabine. Of note, these patients had the lowest CSF darunavir concentrations in their respective groups: 5.79 ng/mL (802 ng/mL in plasma) and 3.47 ng/mL (958 ng/mL in plasma).

Conclusions: Darunavir CSF and plasma concentrations were comparable between the two arms. However, one patient from each group (with the lowest CSF darunavir concentrations in their respective groups) had detectable CSF VL despite undetectable plasma VL.

Keywords: antiretroviral therapy, HIV/AIDS, CSF, ART

Introduction

Despite widespread use of combined ART, neurocognitive impairment is a common event in HIV-1-infected patients.1 Higher penetration of antiretroviral drugs into CSF has been associated with decreased viral replication and an improvement in neurocognitive abnormalities.2

CSF penetration varies between antiretroviral compounds. PIs are large, highly protein-bound molecules, which mainly show restricted CNS penetration.3 However, ritonavir-boosted darunavir (darunavir/ritonavir) and lopinavir reach CSF concentrations above their respective IC50 for WT virus, and may exert an antiviral effect in the CNS.4 Darunavir/ritonavir has considerable antiretroviral potency and a high genetic barrier to resistance.5 The initially approved darunavir/
ritonavir dose was 600/100 mg twice daily, but now a more convenient approved dose of 800/100 mg once daily, which achieves plasma concentrations several-fold greater than the WT IC_{50} (1.78 ng/mL), is widely used in ART-naïve or pretreated patients without previous virological failure to PI-containing regimens.

Many antiretroviral regimens are given at doses that reach plasma concentrations several-fold greater than the WT IC_{50} and are generally well tolerated, but are not free from certain toxicities. Hence, dose reductions may help to decrease adverse effects as well as treatment cost, an important consideration in the current worldwide economic crisis.8,9

Pharmacokinetic/pharmacodynamic analysis of the POWER studies showed that the once-daily darunavir doses of 400 and 800 mg had comparable efficacy in patients with viral strains that were fully susceptible to darunavir, suggesting the possibility of using lower doses of darunavir in such patients.

This study is part of a randomized clinical trial, whose preliminary data suggest that decreasing the darunavir dose from 800 to 600 mg once daily does not compromise efficacy in previously suppressed HIV-infected patients.10 The objective of this substudy was to assess whether a lower dose of darunavir/ritonavir might still achieve good CSF concentrations and contribute to inhibiting CNS viral replication.

Patients and methods

DRV600 is an ongoing randomized, multicentre, open-label study comparing the efficacy and safety of 800/100 mg of darunavir/ritonavir (darunavir 800) versus 600/100 mg of darunavir/ritonavir (darunavir 600) once daily plus two NRTIs in 100 virologically suppressed patients (eudraCT 2011-006272-39).

Paired blood and CSF samples were obtained from each substudy participant 24–28 h after the previous darunavir dose by peripheral venous puncture and lumbar puncture, respectively. Samples were centrifuged and frozen at −70°C until analysis. Total plasma darunavir concentrations were determined by HPLC (lower limit of quantification = 50 ng/mL) and CSF concentrations by liquid chromatography–tandem MS (LC-MS/MS; lower limit of quantification = 2 ng/mL).11,12 HIV-1 RNA viral load (VL) in plasma and CSF (limit of detection = 40 copies/mL) was quantified using a real-time PCR technique (Abbot Molecular USA), performed according to the manufacturer’s recommendations.

Inflammatory and neural damage markers were determined in the CSF of the two patients with viral escape. NFL protein was measured using an NF-light ELISA kit (UmanDiagnóstics AB, Umeå, Sweden) at the Sahlgrenska University Hospital. CXCL13 was measured by ELISA (Human CXCL13/BLC/BCA-1 Immunoassy Kit, R&D Systems Inc., Abingdon, UK). Chitotriosidase was assayed using an in-house enzymatic method according to the procedure described by Hollok et al.13

The study protocol was approved by the ethics committees of the participating hospitals and by the Spanish Drug Agency. All patients gave written informed consent before enrolment.

The results of continuous variables are presented as the median and range, whereas the results of categorical variables are presented as frequencies and percentages. Between-group comparisons used the Mann–Whitney U test for continuous variables and the χ² or Fisher exact test, where appropriate, for qualitative variables. Analyses were performed with SPSS, version 19.0 (IBM SPSS, Chicago, IL, USA).

Results

Sixteen patients (75% males) were included, eight in each arm. The median (range) age was 48 (17–71) years and the median (range) CD4 cell count was 532 (90–1394) cells/mm³. All patients in the darunavir 600 arm and four patients in the darunavir 800 arm received tenofovir/emtricitabine as a part of the antiretroviral regimen, whereas the remaining four patients in the darunavir 800 arm received abacavir/lamivudine.

The median (range) total time on darunavir/ritonavir was 30 (11–57) months, regardless of the study arm. Patients randomized to darunavir 600 had been taking the drug for a median (range) of 10 (7–12) months within the study at the time of sample collection. Similarly, the median (range) time on darunavir 800 within the study was 8 (6–12) months.

Blood and CSF samples were drawn a median (range) of 26 (24–28) h after the last antiretroviral regimen dose. The median (range) darunavir/ritonavir plasma concentrations were 1674 (326–3742) ng/mL in darunavir 600 patients and 1707 (958–3910) ng/mL in darunavir 800 patients (P=0.916). The median (range) darunavir/ritonavir CSF concentrations in darunavir 600 and darunavir 800 patients were 17.08 (5.79–30.19) ng/mL and 13.23 (3.47–32.98) ng/mL, respectively (P=0.916). The median (range) darunavir CSF:plasma ratio was 0.010 (0.005–0.022) in darunavir 600 patients and 0.008 (0.004–0.017) in the darunavir 800 arm (P=0.370).

In 14 patients, CSF VL was <40 copies/mL (Table 1). Of the two patients with detectable CSF VL (280 copies/mL and 159 copies/mL), one was receiving darunavir 600 and the other darunavir 800 plus tenofovir/emtricitabine. Both patients had been taking the treatment correctly and had plasma VL <40 copies/mL. Of note, these patients had the lowest CSF darunavir concentrations in their respective groups: 5.79 ng/mL (802 ng/mL, plasma) and 3.47 ng/mL (958 ng/mL, plasma) (Table 1 and Figure 1). Among markers determined in the CSF of the two patients with viral escape, NFL protein concentrations were normal (350 and 200 ng/L, respectively) and so were the CXCL13 concentrations (<7.8 ng/L in both patients), while CSF chitotriosidase was normal in one patient (2 nkat/L), but was highly abnormal (124 nkat/L) in the patient with a CSF VL of 159 copies/mL.

Discussion

A previous study showed that the widely used 800/100 mg once daily dose of darunavir/ritonavir was associated with lower darunavir concentrations in CSF and lower CSF penetration compared with 600/100 mg of darunavir/ritonavir twice daily.14 In addition, a minority of patients presented very low CSF concentrations of potential concern for HIV control in the central nervous system.5,16 However, despite the low CSF:plasma ratio of darunavir, most of the drug in CSF seems to be unbound, achieving concentrations above the IC_{50} for WT viral strains, and this could contribute to attaining CNS viral suppression even at relatively low doses.5

Our data show that total darunavir concentrations in CSF remain above the drug’s IC_{50} for WT HIV strains (1.78 ng/mL),6 even with the dose reduction to 600 mg once daily. In addition, our findings suggest comparable efficacy in CSF between 800 and 600 mg of darunavir. However, we also observed that, in some patients (i.e. patients 2 and 13), perhaps due to genetic variability or other unknown factors, darunavir concentrations in CSF were close to the IC_{50} and may not have been sufficient to fully inhibit viral replication (Figure 1).

The relevance of inhibiting viral replication in CSF to avoid neurologic damage in HIV-infected patients and to improve the
neurocognitive deficit in symptomatic patients is still controversial. Some authors have found a clinical worsening in patients receiving high penetrating regimens, but others have observed an association between viral escape and HIV-associated neurocognitive disorders, depression and neurological deficits, as well as a greater improvement in neuropsychological performance in patients receiving high CNS-penetrating drugs, and a reduction in inflammatory markers related to viral suppression in CSF.

The finding of viral replication in the CSF of patients receiving triple drug ART containing once daily darunavir/ritonavir highlights the risk of viral escape in patients receiving monotherapy with darunavir/ritonavir that is given with the same darunavir/ritonavir dosage. This regimen is prescribed to selected patients in some countries and is considered an option for switching therapy in special circumstances by the 2014 EACS Guidelines (http://www.easociety.org). Gisslen et al. observed, in two patients receiving 800/100 mg of darunavir/ritonavir once daily monotherapy, viral replication in CSF, associated with an increase in markers of macrophage/microglia activation as well as an increase in NFL protein, a marker of neural damage. In our study, both patients with CSF viral escape had normal values of NFL and CXCL13, but one of them presented highly abnormal values of chitotriosidase, a marker of microglial activation. It is unclear if this implies that a longer time of CSF viral replication is needed for neural damage (NFL) to occur. Another possibility is that detectable VL in CSF sometimes reflects harmless transient peaks and sometimes persistent viral escape that could lead to subsequent symptomatic disease.

**Table 1. Darunavir concentrations and VL in CSF and plasma samples by treatment arm**

<table>
<thead>
<tr>
<th></th>
<th>Plasma darunavir (ng/mL)</th>
<th>CSF darunavir (ng/mL)</th>
<th>CSF:plasma ratio</th>
<th>Plasma VL (copies/mL)</th>
<th>CSF VL (copies/mL)</th>
<th>ART</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>896</td>
<td>16.62</td>
<td>0.019</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>2</td>
<td>802</td>
<td>5.79</td>
<td>0.007</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>3</td>
<td>326</td>
<td>7.14</td>
<td>0.022</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>4</td>
<td>1390</td>
<td>23.04</td>
<td>0.017</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>5</td>
<td>1957</td>
<td>10.56</td>
<td>0.005</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>6</td>
<td>3235</td>
<td>30.19</td>
<td>0.009</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>7</td>
<td>3742</td>
<td>17.54</td>
<td>0.005</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>8</td>
<td>2363</td>
<td>23.99</td>
<td>0.010</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>Median (darunavir 600)</td>
<td>1674</td>
<td>17.08</td>
<td>0.010</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>600/100 of DRV/r + TDF/FTC</td>
</tr>
<tr>
<td>9</td>
<td>1890</td>
<td>11.42</td>
<td>0.006</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>10</td>
<td>1569</td>
<td>13.81</td>
<td>0.009</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>11</td>
<td>3910</td>
<td>32.98</td>
<td>0.008</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>12</td>
<td>1258</td>
<td>17.96</td>
<td>0.014</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>13</td>
<td>958</td>
<td>3.47</td>
<td>0.004</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>14</td>
<td>1876</td>
<td>12.02</td>
<td>0.006</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>15</td>
<td>1845</td>
<td>12.65</td>
<td>0.007</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>16</td>
<td>1389</td>
<td>23.12</td>
<td>0.017</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
<tr>
<td>Median (darunavir 800)</td>
<td>1707</td>
<td>13.23</td>
<td>0.008</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>800/100 of DRV/r + ABC/3TC</td>
</tr>
</tbody>
</table>

DRV/r, darunavir/ritonavir; TDF/FTC, tenofovir/emtricitabine; ABC/3TC, abacavir/lamivudine.

**Figure 1.** Plasma darunavir concentrations and CSF darunavir concentrations by treatment arm. DRV/r, darunavir/ritonavir.
Our study has the limitations of a small sample size and the fact that neurocognitive assessment was not performed. However, it provides relevant data on CSF drug concentrations and viral replication, which contribute to our knowledge on the use of ART in HIV-infected patients. CSF markers were determined only in the two patients with viral escape because this had not been planned before initiation of the study. However, the additional information might be useful to a better understanding of the clinical relevance of presenting viral replication in CSF in patients with viral suppression in blood.

In conclusion, 600/100 mg of darunavir/ritonavir once daily achieves CSF penetration and antiviral activity comparable to those of the 800/100 mg once daily dose. However, with both dosing regimens, some patients may present viral escape in CSF. These data and the consequences of viral replication related to low antiretroviral CSF concentrations should be further evaluated in larger studies.

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