Efficacy of Cerebrospinal Fluid (CSF)–Penetrating Antiretroviral Drugs against HIV in the Neurological Compartment: Different Patterns of Phenotypic Resistance in CSF and Plasma

Andrea Antinori, Carlo Federico Perno, Maria Letizia Giancola, Federica Forbici, Giuseppe Ippolito, Richard M. Hoetelmans, and Stephen C. Piscitelli

1National Institute for Infectious Diseases, “Lazzaro Spallanzani,” Istituto di Ricovero e Cura a CarattereScientifico, Rome, Italy; 2Tibotec-Virco, Mechelen, Belgium; and 3Tibotec-Virco, Raritan, New Jersey

Background. Cerebrospinal fluid (CSF) concentrations of multiple drugs in a large human immunodeficiency virus (HIV)–infected patient population, the virtual phenotype profiles for HIV in the plasma and CSF compartments, and the correlation of these profiles with exposure to antiretroviral therapy need to be further investigated.

Methods. Drug concentrations in CSF and plasma were concomitantly determined for a large group of HIV-infected individuals receiving highly active antiretroviral therapy (HAART). Samples were analyzed using a validated method consisting of liquid chromatography with mass spectrometry. For patients with detectable levels of virus, genotypic analysis was performed, followed by a virtual phenotype study.

Results. Sixty-three HIV-infected patients were included in the study, 78% of whom were affected by neurological disease. Drug concentrations in CSF specimens were undetectable for didanosine, efavirenz, nelfinavir, and concomitantly administered ritonavir and saquinavir. CSF concentrations were higher for nevirapine, with a median CSF-to-plasma concentration ratio of 0.63, followed by lamivudine (0.23), stavudine (0.20), and indinavir (0.11). In 18 of the 40 patients with virtual phenotype data available for virus recovered from CSF samples and from plasma samples, differences in fold-change of resistance between the CSF virus and the plasma virus were noted for at least 1 drug. Factors associated with having differences in fold-change of resistance were number of drugs to which the patient had been exposed (P = .02) and presence of neurological disease (P = .05). A significant association was found between duration of therapy and fold-change of resistance in CSF and plasma isolates.

Conclusions. Antiretrovirals have different levels of penetration in the CSF, with several drugs achieving only low CSF concentrations. CSF isolates have different resistance profiles than do plasma isolates. Effective treatment decisions for CSF manifestations of disease may require better knowledge of drug penetration and the drug susceptibility of HIV in the CSF.

HAART drastically reduces the HIV load in plasma, but residual viral replication may continue in anatomical reservoirs [1]. The CNS is known to be one potential reservoir of HIV infection [2]. HAART has remarkable antiviral activity in the CNS, at least in the case of drug-naive patients. However, after receiving 2 months of potent 4-drug antiretroviral therapy, 36% of drug-naive patients had CSF HIV RNA levels of >50 copies/mL [3], and continuous replication of HIV can continue (even if it is at a low rate), promoting the onset of neurological disorder. A prospective cohort study by Ellis et al. [4] suggested that levels of HIV RNA in the CSF of >200 copies/mL predict the subsequent onset of neuropsychological impairment in HIV-positive patients. Therefore, control of HIV replication in the CSF is an important goal to prevent neurocognitive decline.

The blood-brain barrier reduces delivery of antiretrovirals to the brain. Most antiretroviral drugs penetrate the CSF, but the levels of penetration for a single drug
Figure 1. Protease inhibitor levels in plasma and CSF. F = 16.02 and P < .0001 for CSF levels; F = 3.969 and P = .016 for plasma levels (by analysis of variance). CSF, cerebrospinal fluid; IDV, indinavir; IDV/r, indinavir coadministered with a booster dose of ritonavir; NFV, nelfinavir. RTV, ritonavir; SQV, saquinavir. Boxes, 25th and 75th percentiles; lines, median values; brackets, highest and lowest values.
Table 1. CSF-to-plasma ratios of concentrations of drugs detected in paired samples.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of patients</th>
<th>Median CSF-to-plasma concentration ratio (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abacavir</td>
<td>4</td>
<td>0.039 (0.0–2.36)</td>
</tr>
<tr>
<td>Didanosine</td>
<td>5</td>
<td>0.0 (0.0–0.0)</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>11</td>
<td>0.0 (0.0–0.0)</td>
</tr>
<tr>
<td>Indinavir</td>
<td>18</td>
<td>0.111 (0.0–0.47)</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>55</td>
<td>0.229 (0.0–4.90)</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>9</td>
<td>0.0 (0.0–0.0)</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>16</td>
<td>0.626 (0.41–0.77)</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>8</td>
<td>0.0 (0.0–0.52)</td>
</tr>
<tr>
<td>Stavudine</td>
<td>31</td>
<td>0.204 (0.0–0.204)</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>18</td>
<td>0.02 (0.0–6.74)</td>
</tr>
</tbody>
</table>

RESULTS

Characteristics of patients. Sixty-three HIV-infected patients were included in the study. Patients characteristics at baseline were as follows: 76% of the patients were male; the median age was 39 years (interquartile range, 35–43 years); risk factors for HIV infection were injection drug use for 59% of patients, heterosexual sex for 25%, homosexual or bisexual sex for 10%, and other or unknown for 6%. Thirty-nine patients (62%) had a previous AIDS-defining event. At the time of sample collection, the mean CD4+ cell count was 174 cells/µL (range, 9–729 cells/µL), the mean plasma HIV RNA level was 3.57 log_{10} copies/mL (range, 1.69–6.18 log_{10} copies/mL), and the mean CSF HIV RNA level was 2.58 log_{10} copies/mL (range, 1.90–5.25 log_{10} copies/mL). Twenty-two percent and 49% of subjects had baseline plasma and CSF HIV RNA levels, respectively, less than the detection limit (1.9 log_{10} copies/mL).

A neurological disease was diagnosed in 49 patients (78%); HIV encephalopathy and progressive multifocal leukoencephalopathy were diagnosed in 11 patients each, cytomegalovirus encephalitis was diagnosed in 9, Cryptococcus meningitis and leukoencephalopathy were diagnosed in 4 cases each, toxoplasmic encephalitis was diagnosed in 3, primary CNS lymphoma was diagnosed in 2, and neurological involvement during primary HIV infection or HIV myelopathy was diagnosed in 1 case each. Neurological symptoms in the absence of a specific neurological disorder were present in 3 patients.

With regard to antiretroviral therapy, all subjects had a previous exposure to HAART, with a median duration of exposure preceding lumbar puncture of 325 days (interquartile range, 86–1350 days). A median of 5 drugs (interquartile range, 3–6) had been administered in previous antiviral therapy regimens. Analysis of the specific antiretroviral agents revealed that zidovudine was administered to 82% of patients, with a median duration of exposure of 393 days; didanosine, 40% of patients (median duration, 216 days); zalcitabine, 7% of patients (median duration, 432 days); stavudine, 79% of patients (median duration, 255 days); lamivudine, 98% of patients (median duration, 281 days); nevirapine, 38% of patients (median duration, 126 days); saquinavir, 18% of patients (median duration, 403 days); indinavir, 67% of patients (median duration, 300 days); ritonavir, 27% of patients (median duration, 101 days); nelfinavir, 38% of patients (median duration, 139 days); efavirenz, 22% of patients (median duration, 90 days); abacavir, 7% of patients (median duration, 70 days); amprenavir, 3% of patients (median duration, 100 days); and lopinavir/ritonavir, 5% of patients (median duration, 30 days).

At the time that CSF and plasma samples were collected, patients had been receiving their current HAART regimen for a median of 55 days (interquartile range, 22–180). CSF and plasma samples were collected 0.5–12 h after receipt of the dose.

The drugs included in the most recent antiretroviral regimen were as follows: zidovudine, 22 patients (35%; median duration of exposure, 48 days); didanosine, 8 patients (13%; median duration of exposure, 140 days); stavudine, 38 patients (60%; median duration of exposure, 138 days); lamivudine, 55 patients (87%; median duration of exposure, 103 days); nevirapine, 17 patients (27%; median duration of exposure, 96 days); indinavir, 19 patients (30%; median duration of exposure, 85 days).
days); ritonavir, 8 patients (13%; median duration of exposure, 107 days); indinavir/ritonavir, 4 patients (6%; median duration of exposure, 39 days); saquinavir/ritonavir, 3 patients (5%; median duration of exposure, 337 days); nelfinavir, 14 patients (18%; median duration of exposure, 43 days); efavirenz, 11 patients (17%; median duration of exposure, 49 days); abacavir, 4 patients (6%; median duration of exposure, 20 days); amprenavir, 1 patient (2%; median duration of exposure, 20 days); and lopinavir/ritonavir, 3 patients (5%; median duration of exposure, 30 days).

Pharmacokinetic findings. Drug concentrations were undetectable in the CSF for didanosine, efavirenz, nelfinavir, and concomitantly administered ritonavir and saquinavir for patients receiving these drugs. Only 1 of 8 CSF samples contained detectable levels of ritonavir. As expected, concentrations of protease inhibitors were lower in CSF specimens than in plasma specimens. Levels of specific protease inhibitors were also significantly different in the CSF (F = 16.02; P < .0001, by analysis of variance). In particular, CSF levels of indinavir boosted by ritonavir were higher than levels of either indinavir alone, saquinavir plus ritonavir, or nelfinavir (P < .0001, with Bonferroni’s adjustment) (figure 1). Overall, CSF concentrations were higher for nevirapine, with a median CSF-to-plasma concentration ratio of 0.63 (n = 16), followed by lamivudine (0.23; n = 55), stavudine (0.20; n = 31), and indinavir (0.11; n = 18) (table 1). Although efavirenz was not detectable in CSF, mean CSF HIV RNA levels were lower (albeit not significantly) for patients treated with efavirenz than they were for patients treated with nevirapine. Overall, the number of drugs detectable in the CSF had a linear correlation with the overall number of drugs detectable in plasma (β = 0.536; P < .0001) (figure 2). A significant difference was observed in the mean CSF HIV RNA level only for patients who were treated with at least 1 drug that was detectable in the CSF, compared with patients for whom no drugs were detectable in the CSF (P < .0001, by Student’s t test). The mean plasma HIV RNA level did not significantly differ among patients with 1, 2, 3, 4, or 5 detectable drugs in the plasma.

The CSF HIV load was not significantly correlated with the overall number of drugs detectable in the plasma or in the CSF or with the number of drugs with concentrations greater than the target through levels in CSF. Similarly, the plasma HIV load was not correlated with the total number of drugs greater than the cutoff concentration in plasma.

Virtual phenotypic findings. In 40 patients with a detectable viral load, a virtual phenotype study was performed for both CSF and plasma isolates. For 18 patients, differences in the fold-change of resistance between virus recovered from CSF and virus recovered from plasma were noted for at least 1 drug. Fold-change differences were most common for efavirenz (11 of 18 patients), nevirapine (9 of 18 patients), nelfinavir and stavudine (7 of 18 patients each), and indinavir, amprenavir, and ritonavir (5 of 18 patients each). Factors associated with differences in fold-change resistance were number of drugs to which the patient was exposed (P = .02) and presence of neurological disease (P = .05) (table 2). A significant association was found between duration of therapy and fold-change of resistance in CSF isolates (P = .02, by Spearman’s test) and plasma isolates (P = .002, by Spearman’s test). The total number of drugs susceptible in CSF correlated with the total number of drugs with normal susceptibility range in plasma for patients treated with HAART for >1 year (β = 1.104; P = .02, by linear correlation), but not for patients who were treated for a shorter time. No correlation was observed between the number of drugs susceptible in CSF and the number of drugs greater than the cutoff concentration in CSF.

Table 2. Predictors of virtual phenotypic difference between virus recovered from CSF samples and virus recovered from plasma samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No fold-change difference between CSF virus and plasma virus (n = 22)</th>
<th>At least 1 fold-change difference between CSF virus and plasma virus (n = 18)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV RNA level, log_{10} copies/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>5.38</td>
<td>5.25</td>
<td>.73</td>
</tr>
<tr>
<td>Plasma</td>
<td>5.48</td>
<td>5.17</td>
<td>.34</td>
</tr>
<tr>
<td>Plasma-to-CSF concentration ratio</td>
<td>1.29</td>
<td>1.31</td>
<td>.88</td>
</tr>
<tr>
<td>Exposure to antiretroviral therapy, % of patients</td>
<td>36</td>
<td>72</td>
<td>.03</td>
</tr>
<tr>
<td>Duration of antiretroviral therapy, years</td>
<td>1.18</td>
<td>1.43</td>
<td>.73</td>
</tr>
<tr>
<td>No. of drugs in treatment regimen</td>
<td>4.0</td>
<td>5.8</td>
<td>.02</td>
</tr>
<tr>
<td>Duration of PI therapy, days</td>
<td>170</td>
<td>462</td>
<td>.003</td>
</tr>
<tr>
<td>Duration of NRTI therapy, days</td>
<td>433</td>
<td>578</td>
<td>.58</td>
</tr>
<tr>
<td>Neurological disease, % of patients</td>
<td>68</td>
<td>94</td>
<td>.05</td>
</tr>
</tbody>
</table>

NOTE. PI, protease inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor.
A negative correlation between the number of susceptible drugs observed in virtual phenotypic analysis and the number of years of exposure to antiretroviral therapy was observed for both plasma isolates ($\beta = -1.085; P = .016$) and CSF isolates ($\beta = -1.209; P = .028$). No significant correlation between viral loads (in plasma and in the CSF) and virtual phenotypic susceptibility score was found.

**DISCUSSION**

This study represents the largest study of collected, paired CSF and plasma samples in HIV-infected subjects who are receiving HAART. Many studies have reported the pharmacokinetic profiles of drugs and the penetration of a single antiretroviral in the CSF [5–15]. In the present study, we assessed CSF concentrations for multiple drugs in a large population of HIV-infected patients. We also investigated the profiles resulting from a virtual phenotypic study of the 2 compartments (i.e., plasma and the CSF) and their correlation with exposure to antiretroviral therapy.

Previous articles revealed that the use of CSF-penetrating drugs substantially enhances antiviral response to HAART in the CSF [19–21]. In our experience, for a group of patients with advanced disease who were affected by neurological disorders, a significant difference in the magnitude of reduction in the CSF HIV RNA level was observed among recipients of $\geq 3$ drugs that penetrate the blood-brain barrier [19].

Antiviral therapy failure can occur for several reasons, including poor adherence to the treatment regimen, insufficient drug potency, the emergence of drug-resistant virus, and pharmacokinetic factors [22]. Determination of drug levels is useful for evaluation of inadequate drug exposure, interpatient variability, and antiretroviral drug-drug interaction. Several published studies have evaluated the correlations between plasma drug levels, resistance, and virological efficacy, but no definitive conclusions have been made [23–28]. Some prospective analyses have shown that use of “optimal” drug concentration information, when combined with resistance test data, is associated with a better rate of therapeutic success both for treatment-naive patients (the Athena study [23]) and for salvage therapy (the Viradap study [24] and the Terry Beirn Community Programs for Clinical Research on AIDS 046 study [25]). However, other prospective randomized studies of antiretroviral-experienced patients, such as the PharmAdapt study [26] and the GENOPHAR study [27], found no statistically significant difference in virological outcome between the therapeutic drug monitoring arm, in which therapy was modified using genotypic resistance testing data combined with plasma levels, and the control arm, for which only genotypic testing data were used. Recently, the Resistance and Dosage Adapted Regimens study failed to support the use of concentration-controlled intervention in salvage therapy [28].

The efficacy of phenotypic resistance testing for optimizing the antiretroviral treatment is still controversial [29, 30]. By combining data on plasma drug levels with phenotypic resistance data, it is possible to assess whether the individual patient’s drug exposure is sufficient to overcome the patient’s viral resistance pattern.

Phenotypes of CSF and plasma isolates have been studied in structured treatment interruptions after virological failure [31]. In that study, phenotypic susceptibility profiles were nearly identical for isolates in paired CSF and plasma samples at baseline and throughout the course of structured treatment interruption.

In the present study, we observed a broad variability of drug concentrations in the CSF. Many drugs that are commonly used in clinical practice (e.g., didanosine, efavirenz, nelfinavir, ritonavir, and ritonavir plus saquinavir) were almost always undetectable in the CSF. On the contrary, the highest drug levels in the CSF and plasma have been found for protease inhibitors during concomitant administration of indinavir and ritonavir and for nevirapine of the 2 nonnucleoside reverse-transcriptase inhibitors. This finding is to be expected, because indinavir and nevirapine have the lowest plasma protein-binding ability of antiretroviral drugs in their class, and protein binding is a major determinant of CSF penetration. Notably, efavirenz was not detected in any CSF sample. The effect of efavirenz in controlling HIV replication in microglia is well documented in vitro [32]. However, the mean CSF HIV RNA level for patients treated with efavirenz was not significantly less than that for patients treated with nevirapine. Furthermore, clinical studies have demonstrated that virological failure was observed in only 50% of subjects who received efavirenz in combination therapy and for whom drug concentrations were suboptimal [33], and that efavirenz used in combination therapy had low CSF concentrations but is effective in suppressing the CSF HIV RNA level [14, 34].

Characteristics of HIV infection in CSF are studied as a surrogate for such characteristics in the brain; drug concentrations can be detected in that fluid. However, drug levels in biological fluids cannot be used as direct markers of efficacy for multiple reasons. First, CSF penetration may not be necessarily equivalent to parenchymal penetration of available antiretroviral drugs. Second, drug levels in the CSF may have little relationship to drug levels in brain extracellular fluid [35], and the effect in CSF can be different from that in brain parenchyma. Indeed, brain extracellular fluid drug levels, rather than CSF drug levels, may be most crucial for suppression of CNS viral replication. Moreover, protease inhibitors may be actively eliminated from the brain by active efflux transporters, as demonstrated in vitro for multidrug resistance protein P-glycoproteins, such as human multidrug resistance protein 2, which efficiently transports saquinavir, ritonavir, and indinavir [36].
Nucleoside analogues require intracellular phosphorylation of their active triphosphates, and plasma concentrations do not necessarily reflect the intracellular amount of pharmacologically active triphosphates. However, despite these limitations, drug concentrations in the CSF still represent a major factor to be taken into consideration for the evaluation of the presence and efficacy of antiviral drugs in the CNS.

A potential limitation of the present study could be that single samples were obtained only once for individual patients. Some experts consider this approach as unrepresentative because of high intrapatient variability. However, all samples were collected under the same conditions. Another limitation is that the time of collection and time of dosing were not standardized, but to do these would be impossible. Some may comment that perhaps all samples were collected when drug concentrations were at trough levels and that this would explain low concentrations in CSF. The virtual phenotype study part of our work has shown that different resistance profiles occur for at least 1 drug in CSF isolates (compared with plasma isolates) in 45% of HIV-infected patients. In a previous report, Price et al. [31] also reported a high rate of phenotypic resistance in HIV from paired CSF and plasma samples obtained from 5 patients who interrupted therapy.

The duration of therapy was significantly associated with the emergence of virtual phenotypic resistance, outlining the risk that long-term antiretroviral therapy could favor the onset of resistance and subsequent virological failure, especially if adherence to the treatment regimen is not good. Neurological disorders were correlated with the presence of virtual phenotypic resistance. This observation suggests that, at the time of onset of clinically detectable neurological disease, the neurological compartment is autonomous. In conclusion, our finding of different profiles of resistance between plasma and CSF isolates supports the hypothesis that CSF and plasma are 2 virologically distinct compartments, as was previously suggested [17, 18], and it underlines the necessity of attaining complete control of HIV replication in the CSF as well as in plasma. In the case of virological failure, it could be useful to investigate the CSF compartment to look for potential resistance.

Integration of virological and pharmacological characteristics could provide a better means to predict virological response and can be helpful in determining therapeutic strategies, especially for patients with a neurological impairment. The decreased ability of some drugs to achieve adequate concentrations in the CSF and the resistance profile in the CSF may need to be considered, especially if CNS manifestations are present. Treatment decisions for CSF manifestations may require knowledge of drug penetration and the susceptibility of HIV in CSF, but the optimal antiretroviral regimen for treating HIV infection in the cerebral compartment has not yet been defined.