Therapy Failure following Selection of Enfuvirtide-Resistant HIV-1 in Cerebrospinal Fluid

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We report the selection of enfuvirtide-resistant human immunodeficiency virus type 1 in cerebrospinal fluid, resulting in subsequent loss of viral suppression in the plasma. This case report emphasizes the potential danger of low-level penetration of entry inhibitors into the central nervous system.

Currently, 2 antiretroviral agents interfering with human immunodeficiency virus (HIV)–1 entry, enfuvirtide and maraviroc, are available for treatment of patients infected with multidrug-resistant HIV-1. Limited data are available regarding the penetration of these drugs into the central nervous system (CNS), a potential sanctuary site of HIV [1]. We describe selection of enfuvirtide-resistant HIV-1 in the cerebrospinal fluid (CSF) of a patient with undetectable plasma HIV RNA, ultimately resulting in therapy failure.

Case report. In 2007, a 50-year-old Caucasian man presented with sensory disturbances and unsteadiness of the lower extremities leading to an uncoordinated gait. He had been diagnosed with HIV-1 infection in 1994 for which he was initially treated with zidovudine monotherapy. During the years thereafter, several treatment changes were made because of virological failure and toxicity, with subsequent selection of multidrug-resistant HIV. The adverse effects subsided after each change in his antiretroviral regimen.

In 2005, the CD4+ cell count was 38 cells/mm³, and the plasma HIV RNA level was >750,000 copies/mL. Genotypic resistance analysis (sample P1) (Figure 1A) identified multiple resistance mutations in reverse transcriptase (M41L, E44D, D67N, L74V, A98G, K101H, V188I, Y188L, M184V, L210W, T215Y, K219E, and H221Y) and protease (L10I, I13V, L33F, I54V, L63P, A71V, V77I, V82S, 184V, and L90M) but no enfuvirtide-related mutations [2]. Tropism testing (The Original Trolife Assay; OTA, Monogram Biosciences) reported presence of CCR5-tropic virus only.

Salvage therapy was subsequently started containing maraviroc, enfuvirtide, tenofovir, zidovudine, and lamivudine (Figure 1A). On the basis of a resistance interpretation algorithm [3], maraviroc and enfuvirtide were the only fully active drugs in this regimen.

Immunologic recovery was rapidly achieved; the CD4+ cell count increased to 579 cells/mm³. However, virological response was slow; after an initial response of >3-log decrease, prolonged low-level replication was observed before the HIV RNA level was suppressed (<50 copies/mL) (Figure 1A).

Soon after initiation of the regimen, sensory disturbances of the feet occurred, followed by progressive gait problems 20 months later. Neurological examination revealed a spastic gait, exaggerated knee reflexes, absent ankle reflexes, and plantar reflexes according to Babinski. Sense of pain, temperature, touch, pressure, vibration, and joint position were impaired in the lower extremities.

Laboratory examinations reported macrocytic anemia (hemoglobin concentration, 8.1 mmol/L; mean corpuscular volume, 115 fl), which was compatible with zidovudine use and/or slight folic acid (level, 5.8 nmol/mL) and vitamin B12 (level, 110 pmol/L) deficiency. Thoracic and lumbar spine magnetic resonance imaging revealed no abnormalities. Electromyography was undisturbed; however, tibial nerve somatosensory evoked potentials showed prolonged conduction times, compatible with myelopathy. A CSF specimen revealed an elevated leukocyte count (48 leukocytes/mm³), a slightly increased protein level (0.58 g/L), and a normal glucose level (3.9 mmol/L). Infection with Treponema pallidum, Borrelia burgdorferi, human T lymphotropic virus type 1, herpes simplex virus, and varicella zoster virus was excluded. On the basis of these results, subacute combined degeneration of the spinal cord due to vitamin B12 deficiency was recognized as the most likely cause of the patient’s neurological symptoms, and therapy with folic acid and vitamin B12 was initiated.

However, although HIV RNA was undetectable in the concurrent plasma sample, analysis of the CSF demonstrated an HIV RNA load of 2780 copies/mL (sample CSF1) (Figure 1A).
Figure 1. A, Plasma and cerebrospinal fluid (CSF) human immunodeficiency virus (HIV) RNA levels after the start of salvage regimen (bars). B, Phylogenetic tree of HIV-V3 sequences in CSF and plasma samples. P1–P7 correspond to the different plasma samples, and CSF1–CSF3 correspond to the different CSF samples. P2 and P6 could not be included in the phylogenetic tree because of insufficient material. Dosages were as follows: lamivudine (150 mg) plus zidovudine (300 mg) twice per day, tenofovir (245 mg twice per day), enfuvirtide (90 mg twice per day), maraviroc (300 mg twice per day, changed to 150 mg twice per day when darunavir was started), darunavir (600 mg twice per day; boosted by ritonavir), and emtricitabine (200 mg once per day).

Therefore active viral replication in the CNS as possible cause of his symptoms could not be excluded either.

To differentiate between active viral replication in CSF and viral release by infected cells only, the protease inhibitor darunavir-ritonavir, which just had become available, was added. Within 4 weeks after this intervention, the CSF HIV RNA load became undetectable (<250 copies/mL), indicating suppression of active viral replication (sample CSF3) (figure 1A). Despite suppression of HIV RNA in CSF and correction of the vitamin B12 level, the patient’s neurological symptoms did not significantly improve. To eliminate potential neurotoxic effects, zidovudine was discontinued and lamivudine was switched to emtricitabine. Subsequently, the patient’s sensory disturbances disappeared slowly, although his gait problems persisted. In 2008, ten months after addition of darunavir-ritonavir to the patient’s regimen, the plasma HIV RNA level rebounded to 270 copies/mL (sample P7) (Figure 1A). We questioned whether viral replication in CSF was related to therapy failure and set out in-depth investigations.

Methods. The patient participates in the ATHENA observational cohort, which was approved by the national institutional review board.

Longitudinal plasma and CSF samples were analyzed (Figure 1A). Population genotypic analysis was performed (protease, RT, env gp41, and gp120-V3) [4,5]. Gp41 was amplified and sequenced (BigDye Terminator Cycle Sequencing kit; Applied Biosystems) using forward primer FRgp41F (GCWGGAAGCACAAGCGC) and reverse primer FRgp41R (TGTARTACCC-
A large-volume (4.3-mL) input was used to perform ultrasensitive analysis on plasma samples positive for HIV RNA with levels below quantification (<50 copies/mL). V3-sequences were aligned to a reference from the Los Alamos database (http://www.hiv-web.lanl.gov) using Clustal software (http://megasoftware.net). Neighbor-joining phylogenetic trees were constructed using Megav4.1 (Beta) software (http://megasoftware.net) and the maximum composite likelihood model and γ-distributed rates (γ parameter, 0.5). Bootstrap analysis was performed using the same methods (1000 replicates). Bootstrap values >70% were interpreted as support for clustering.

Drug levels were measured by liquid chromatography tandem mass spectrometry. Total enfuvirtide and maraviroc levels were measured in CSF and plasma specimens 3 and 1.5 h after drug intake, and the total darunavir level was measured in CSF and plasma specimens 2.5 and 3.75 h after drug intake.

Results. Genotypic analysis of CSF-derived HIV RNA did not reveal changes at resistance-related positions in protease and reverse transcriptase, compared with the baseline plasma sample (samples CSF1-2 and P1) (Figure 1A) [2]. In addition, CSF sequence analysis of the env-gp120 V3-loop showed no differences from baseline, suggesting no change in HIV tropism. Interestingly, analysis of the heptad repeat regions of env-gp41 revealed the enfuvirtide-related V38A mutation in the CSF (sample CSF2) (Figure 1A), whereas ultrasensitive resistance analysis in the concurrent plasma sample (HIV RNA level <50 copies/mL (~25 copies/mL)) did not show selection of enfuvirtide mutations (sample P6) (Figure 1A). In addition, retrospective analysis of a sample obtained during the period of low-level replication after initiation of the regimen (HIV RNA level, 439 copies/mL) did not reveal enfuvirtide mutations (sample P2) (Figure 1A).

Subsequently, drug levels were measured to relate the differential selection of antiviral resistance in the 2 compartments to differences in drug pressure. Total enfuvirtide concentrations of 3.74 and 0.055 μg/mL were measured in plasma and CSF, respectively. On the basis of plasma protein binding of 92%, the enfuvirtide concentration in CSF appeared to be 5.5-fold lower than the estimated free plasma concentration of 0.29 μg/mL [6]. The total plasma concentration of maraviroc was 0.146 μg/mL, corresponding to a free plasma concentration of 0.04 μg/mL (76% protein binding [7]), whereas only traces of maraviroc below the limit of quantification (0.039 μg/mL) could be detected in the CSF. The plasma level of darunavir was 3.3 μg/mL, corresponding to ~0.17 μg/mL of free darunavir (95% protein binding [8]). The darunavir concentration in CSF was estimated to be 0.019 μg/mL, which is 9-fold lower than the calculated free plasma concentration and is in line with recently published results [9].

At time of therapy failure 11 months later (sample P7) (Figure 1A), genotypic analysis detected, for the first time, the V38A mutation in plasma. Phylogenetic analysis demonstrated a close relationship (bootstrap value, 93%) of this rebound plasma population with the viral population in the CSF sample (samples P7 and CSF2-3) (Figure 1B).

Discussion. In this case report, we describe selection of enfuvirtide-resistant HIV in CSF during salvage therapy while HIV RNA in the plasma was suppressed below the limit of quantification. Subsequent addition of boosted darunavir to the antiretroviral regimen resulted in suppression of HIV RNA levels in the CSF. Although the CSF concentration of darunavir was low, it was still in the range of the 50% inhibitory concentration of darunavir–susceptible virus (0.012–0.055 μg/mL) [9]. Investigations into CSF penetration of another boosted protease inhibitor also suggest that reduction of CSF HIV RNA levels can be observed if CSF drug levels exceed the 50% inhibitory concentration for wild-type virus [10].

Currently, only limited data regarding the penetration of entry inhibitors into the CNS have been published. In this patient, we observed poor CSF penetration of maraviroc, in line with data of animal studies [11]. To our knowledge, these are the first data published on CSF maraviroc concentrations in humans. Recently, negligible penetration of enfuvirtide in CSF and presence of enfuvirtide-resistant HIV in CSF was reported in a patient with a transient increase in the CSF HIV RNA level (while HIV RNA was detectable in plasma) [12]. We demonstrate that viral replication resulting in selection of enfuvirtide-resistant HIV-1 in the CSF can precede selection of enfuvirtide-resistant virus in the plasma.

One could argue that enfuvirtide resistance was already selected in the plasma during the initial slow decay after start of salvage therapy. In our opinion, this scenario is unlikely, because we did not detect the V38A mutation in plasma at this point in time. Furthermore, at time of detection of the V38A mutation in CSF, no enfuvirtide resistance was observed in the concurrent plasma sample with an undetectable viral load using ultrasensitive analysis.

Therefore, the most likely explanation is that low CSF enfuvirtide concentrations (in the absence of adequate maraviroc levels) enabled viral replication in the CSF, leading to subsequent selection of enfuvirtide drug-resistance. Enfuvirtide-resistant HIV eventually appeared in blood, possibly via migration of replication competent cells or altered blood-brain barrier permeability, resulting in therapy failure. This suggests that treatment with enfuvirtide should be combined with at least 1 active antiretroviral drug with adequate CNS penetration.

To our knowledge, this is the first case describing therapy failure after initial selection of resistant HIV-1 in a sanctuary site, illustrating the risk of low-level penetration of antiretroviral drugs in the CNS.
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