Journal of NeuroVirology, 10(suppl. 1): 52–57, 2004

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280490270824

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Long-term virological effect of highly active antiretroviral therapy on cerebrospinal fluid and relationship with genotypic resistance

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The objective of this study was to assess the long-term virological response in cerebrospinal fluid (CSF) in patients treated with highly active antiretroviral therapy (HAART) and to compare this response to CSF and plasma human immunodeficiency virus (HIV) drug resistance profiles. Paired CSF and plasma specimens were drawn from 18 patients receiving HAART at baseline and after 9 to 70 months of therapy. At baseline, median HIV-1 RNA concentrations were 4.13 $\log_{10}$ copies/ml in CSF and 5.31 $\log_{10}$ copies/ml in plasma. At the time of on-therapy CSF sampling, HIV-1 RNA was undetectable in CSF from 13/18 patients (72%), and in plasma from 9/18 patients (50%). The genotypic analysis at baseline revealed reverse transcriptase (RT) resistance mutations in 7 of 11 (64%) CSF samples and in 8 of 11 (73%) plasma samples. No patient had protease resistance mutations, except for secondary mutations. At the time of virological failure in CSF, new RT and protease resistance mutations were found in both CSF and plasma of the two patients with both baseline and on-treatment paired evaluations. At long-term follow-up, the proportion of patients failing to respond virologically was lower in CSF than in plasma. Virological failure in CSF was associated with failure to respond in plasma and onset of new drug resistance mutations in both compartments. *Journal of NeuroVirology* (2004) **10(suppl. 1),** 52–57.

Keywords: cerebrospinal fluid; HAART; resistance mutations

Introduction

During the years following the advent of highly active antiretroviral therapy (HAART), a body of information has been collected on the effect of these treatments on human immunodeficiency virus (HIV) infection of the central nervous system (CNS). HAART has been associated with a decline of both incidence and prevalence of acquired immunodeficiency virus (AIDS) dementia complex (ADC) in cohort studies (Dore *et al*, 1999; Sacktor *et al*, 2001). Furthermore, both clinical and neuroradiological improvement is usually observed in patients with ADC or milder HIV-related cognitive deficits after a few weeks of HAART. Virologically, HAART is usually followed by a decrease in cerebrospinal fluid (CSF) HIV-1 RNA levels (Gisslen *et al*, 1997). Like in plasma, this effect is evident as soon as during the first days of therapy; however, the dynamics of viral load decay in CSF may differ from that in plasma. Actually, two distinct patterns of response have been observed: a first pattern, consisting in a parallel decrease of CSF and plasma HIV-1 RNA levels, and a second, characterized by a slower decline in CSF compared to plasma (Ellis *et al*, 2000; Staprans *et al*, 1999). It is likely that, in the first case, the CSF virus derives predominantly from peripheral cells trafficking within the CSF. In contrast, the CSF virus might originate intrathecally in the latter case, being produced by brain mononuclear phagocytes (Price, 2000). In the case of productive brain infection, CNS penetration of individual drugs, as well as specific properties of brain

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Received 27 February 2003; accepted 30 May 2003.

macrophages, involving drug metabolism and virus turnover, might influence the response to HAART.

It has been hypothesized that, because of the poor penetration of some antiviral drugs in the CNS, viral replication could be suppressed less efficiently in this tissue than in plasma, thus creating the ground for local virus rebound and development of resistant HIV strains. Long-term treatment with HAART is associated with a high proportion of virological failure in plasma. However, preliminary observations suggest that virological failure in CSF might occur less frequently than expected compared to plasma.

The aim of this study was to study the long-term response to HAART in both CSF and plasma samples drawn at HAART baseline and after long-term treatment follow-up. The viral load was compared to the resistance profile for reverse transcriptase (RT) and protease in both compartments. Finally, relevant published studies analyzing the virological response in CSF compared to resistance patterns in CSF and plasma were briefly reviewed.

Results

The changes in CSF and plasma HIV-1 RNA levels were evaluated in 18 patients treated with HAART. Eight of these patients had on-treatment viral load assessed at two different time points, giving a total of 26 measurements. At the time of the most distant viral load assessment from baseline, median time on HAART was 19 months (range 9–70 months).

Viral load changes from baseline are shown in Figure 1. At baseline, median HIV-1 RNA concentrations were 4.13 $\log_{10}$ copies/ml in CSF and 5.31 $\log_{10}$ copies/ml in plasma. During therapy, HIV-1 RNA became undetectable in plasma from 9/18 patients (50%) and in CSF from 13/18 patients (72%). Overall, 17 of 26 CSF (65%) and 11 of 26 plasma samples (42%) drawn during the follow-up had undetectable HIV-1 RNA levels. Four patients failed to respond in both CSF and plasma, four patients in plasma only and one patient in CSF only. Patients failing to respond in both compartments always had plasma viral levels higher than in CSF. In the patient failing to respond in CSF only, CSF HIV-1 RNA load was 3.65 $\log_{10}$ copies/ml, but at the time of failure, he was receiving a combination of two protease inhibitors, consisting of lopinavir/ritonavir and saquinavir.

The pattern of genotypic resistance for RT and protease was evaluated in CSF and plasma of all the patients from whom sufficient amount of sample was available and/or HIV-1 RNA levels were sufficient to allow RNA amplification from the RT and/or protease genes. At baseline, the genotypic analysis revealed RT resistance mutations in 7 of 11 (64%) plasma samples and in 8 of 11 (73%) CSF samples. No patient had protease resistance mutations, except for secondary mutations. At this time discordant resistance profiles between CSF and plasma were found in 5 of 10 patients with paired analyses, for a total of 13 discordant mutations out of a total of 46 mutations identified. Discordances were more similarly frequent in CSF (8 of 24) than in plasma (6 of 22). Plasma samples taken during follow-up disclosed resistance mutations in all of four samples examined. New resistance mutations were found in both plasma and CSF from the two patients with both baseline and on-treatment determinations (Table 1, patients 9, 11). Different mutations between CSF and plasma were found in one of these patients.

Discussion

Concern has been risen around the hypothesis that HAART could lead to incomplete suppression of viral replication in the CNS, due to the potential limited penetration of some antiretroviral drugs through the brain barriers. Such occurrence would have important implications in the CNS, where elevated viral replication levels can be associated with HIV-related tissue damage and neurological disease (Wiley and Achim, 1994). Furthermore, persistent virus

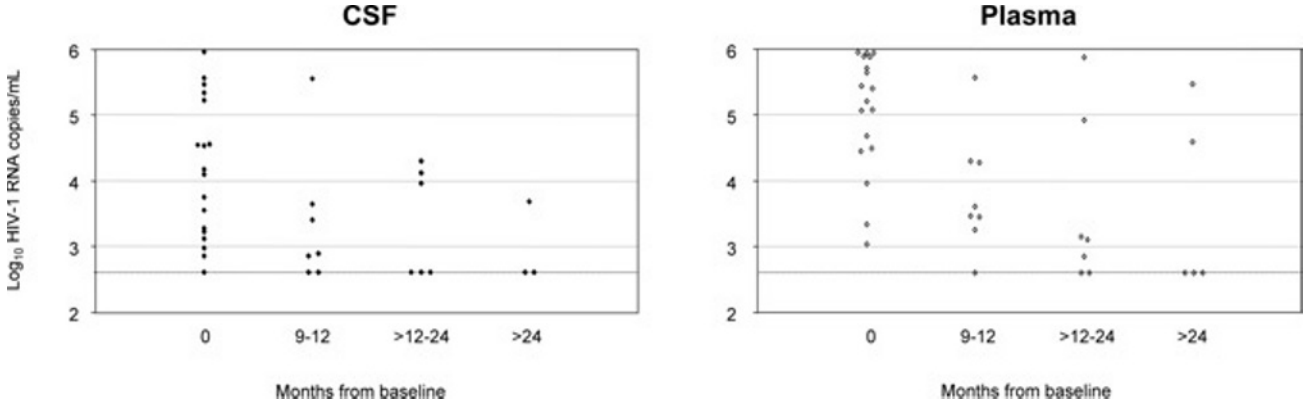

Figure 1 Cerebrospinal fluid and plasma HIV-1 RNA load in 18 HAART-treated patients. Both levels at baseline and during treatment (one or two assessments) are shown for each patient. The dotted line indicates the cut-off value, set at 400 copies/ml.

Table 1 CSF and plasma HIV-1 levels and reverse transcriptase and protease resistance mutations at baseline and after >9 months of therapy

Patient	*Months on therapy*	*Treatment*	*CSF HIV-1 RNA (log copies/ml)*	*CSF reverse transcriptase mutated codons*	*CSF protease mutated codons*	*Plasma HIV-1 RNA (log copies/ml)*	*Plasma reverse transcriptase mutated codons*	*Plasma protease mutated codons*
HAART baseline								
1		AZT, 3TC, IDV	2.61	n.d.	n.d.	5.41	74, 98, 184	36, 63
2		AZT, 3TC, IDV	2.98	69, 70, 230	n.d.	3.04	75	n.d.
3		3TC, d4T, IDV	3.11	41, 215	n.d.	4.45	w-t	n.d.
4		3TC, d4T, NFV	3.23	179	n.d.	3.95	74	n.d.
5		3TC, d4T, SQV	3.55	67, 70, 184, 215, 219	n.d.	3.34	n.d.	n.d.
6		3TC, d4T, IDV	3.75	w-t	w-t	5.89	w-t	n.d.
7		AZT, 3TC, RTV	4.18	98, 101, 106, 108	n.d.	4.69	98, 101, 106, 108	n.d.
8		AZT, 3TC, RTV	4.54	41, 98, 215, 227	63	5.43	41, 44, 98, 118, 210, 215	36, 60, 63
9		3TC, d4T, IDV	4.54	w-t	63	>6.00	w-t	63
10		3TC, d4T, IDV	5.23	184	63	5.64	184	10, 63
11		AZT, 3TC, IDV	6.34	41, 44, 67, 98, 108, 184, 210, 215, 219	20, 63	>6.00	41, 44, 67, 98, 108, 118, 210, 215, 219	63
12		3TC, d4T, IDV	5.46	w-t	10, 63	5.90	w-t	10, 63
During HAART								
1a	9	AZT, 3TC, IDV	3.40	74, 98, 184	36, 63	3.45	74, 98, 184	36, 63
1b	15	AZT, 3TC, IDV	3.96	74, 98, 184	36, 82	3.10	74, 98, 184	n.d.
9	18	3TC, d4T, NFV	4.13	184	36, 63	4.92	184	36, 63
10a	9	3TC, d4T, IDV	<2.60	n.d.	n.d.	3.61	184	63
10b	11	3TC, d4T, IDV	<2.60	n.d.	n.d.	4.30	184	n.d.
11a*	10	AZT, 3TC, IDV	5.56	41, 44, 67, 98, 108, 118, 184, 210, 215, 219	10, 46, 63, 71, 73, 77, 82, 90	5.57	41, 44, 67, 98, 108, 118, 184, 210, 215	10, 46, 63, 73, 77, 90
11b	19	d4T, SQV, NFV	4.29	41, 44, 67, 98, 103, 108, 118, 210, 215	10, 20, 46, 47, 53, 54, 63, 71, 73, 82, 90	5.87	41, 44, 67, 98, 100, 103, 108, 118, 210, 215, 219	10, 20, 46, 53, 54, 63, 71, 73, 82, 90

Note. Only patients in whom genotypic resistance was assessed at least once (at baseline alone or at both baseline and follow-up) are presented. Treatment at baseline refers to HAART after baseline. Treatment during HAART refers to last drug combination.
n.d., not deterrmined; w-t, wild-type; AZT, zidovudine; 3TC, lamivudine; d4T, stavudine; RTV, ritonavir; IDV, indinavir; SQV, saquinavir; NFV, nelfinavir.
*Plasma sampling preceeded CSF sampling by 1 month.

replication in the CNS in the presence of suboptimal drug levels might theoretically predispose to local development of viral resistance to antiviral drugs. Because of the continuous trafficking of immune cells between CSF and plasma, such resistant virus might eventually spread from the CNS to the periphery.

There are at present some observational data supporting the hypothesis that resistant strains can develop selectively in the CNS. In this regard, several studies comparing CSF and plasma sequences have shown different resistant mutations in the two compartments, with mutations found in CSF but not in plasma in up to one third of the cases (Chien *et al*, 1999; Cunningham *et al*, 2000; Venturi *et al*, 2000). The majority of these studies, however, were cross-sectional and they were not designed to address questions such as the dynamics of onset of resistance in the two compartments, the correlation between resistance mutations and viral replication in the two compartments, or with development of HIV-induced CNS disease.

Only few studies have analyzed RT and protease resistance in paired CSF and plasma samples collected longitudinally from HAART-treated patients. In the majority of these studies, however, resistance mutations were assessed only at baseline or during the first days to weeks from the start of an effective HAART regimen, when sufficient amounts of viral RNA was still detectable. Tang *et al* (2000) showed that the viral CSF sequences at baseline did not differ from CSF sequences drawn during treatment, whereas the sequences in CSF samples differed significantly from those in plasma. We also previously shown the persistence of same resistance profiles in CSF and plasma samples during the first phases of a successful HAART (Cinque *et al*, 2001). In the same study, we failed to demonstrate a consistent correlation between presence and pattern of resistance mutations at baseline and the kinetics of virological response in the two compartments during the first weeks of HAART.

Patients who fail to respond to HAART would be the natural population in whom to address the issues of the possible CNS selection of viral resistant mutants and of its clinical implications. Virological rebound following an initial response to therapy is increasingly frequent in HAART-treated patients, especially in those treated for long periods. Virological failure may result from a number of factors, primarily suboptimal adherence to therapy and development of resistance due to nonsuppressing regimens. There are a few, preliminary studies reporting on the long-term response to HAART in CSF. Staprans *et al* (1999) described a few patients treated for up to 37 weeks, observing a continuing and gradual response in CSF despite incomplete plasma response. These authors suggested that more prolonged inhibition of viral replication in CSF than in plasma can reflect the slower virus turnover in brain macrophages, which was also likely responsible for the slow response in CSF observed during the first treatment period. These observations are corroborated by a study from Gunthard *et al* (2001), who searched for the presence of residual virus replication in potential sanctuary body sites in patients receiving HAART for more than 2 years. The authors found that lymph nodes continued harboring infectious virus, but HIV-1 RNA was never detected in the CSF, leading them to conclude that the CNS is unlikely to be the major source of viral rebound under potent antiretroviral therapy.

A more recent study conducted on children receiving a combination of either two or three nucleoside RT inhibitors (NRTIs; zidovudine and lamivudine *versus* zidovudine, lamivudine and abacavir) demonstrated an overall high proportion of virological failure in CSF, of almost 50% at week 48. This response, however, was less marked in CSF than in plasma (McCoig *et al*, 2002). In four children, CSF and plasma pairs were available both at baseline and after 48 weeks of HAART, showing a discordant evolution of HIV between the two compartments. In three of these four cases, new zidovudine resistance-associated mutations were observed in CSF but not in plasma. Despite these findings, however, a remarkable improvement was observed in all of the children with neuropsychological impairment.

Our study investigated the long-term response to HAART in a group of patients with advanced HIV infection and neurological problems. These patients, in theory, are those more at risk for experiencing a viral rebound in CNS and also develop active CNS disease by HIV. The length of treatment varied among patients, and several patients changed repeatedly their HAART before last follow-up. Overall, 50% of the patients failed to respond virologically in plasma. Only half of these, however, also failed to respond in CSF. In only one patient was the virus detectable in CSF but not in plasma. Most likely, the treatment regimen in this patient, consisting of a two-protease inhibitor combination, was the reason for the selective failure to respond in CSF. It has actually been shown that the combination of ritonavir and saquinavir is ineffective to reduce CSF viral load until undetectable levels after 12 weeks, as compared to a combination of the same drugs plus stavudine (Gisolf *et al*, 2000).

As expected, all the patients who failed to respond virologically in plasma had RT and/or protease resistance mutations. Patients failing to respond in CSF also had resistance mutations in this compartment. Discordant resistance mutations between CSF and plasma at this time were found in one case, suggesting a different evolution of viral strains in the two compartments. In particular, the protease mutation at codon 82 was present in CSF after 10 months of HAART, but it was not revealed in a plasma specimen taken one month earlier. Although remarkable, these findings cannot prove transmission of resistant virus from the CSF to the blood. In general, the potential transmission of resistant virus from one compartment to the other is difficult to demonstrate. Intensive

serial CSF and plasma sampling may actually provide the only means to study the kinetics of emergence of resistance mutations and the interactions with viral replication in the two compartments.

Finally, in none of our patients was viral rebound in CSF associated with onset of HIV-related CNS disease. Only one patient in this series had a diagnosis of ADC before starting HAART. Clinically, he only partially improved while on therapy, although his viral load became undetectable from both CSF and plasma.

In summary, our study shows that long-term HAART is accompanied by a proportionally better virological response in CSF than in plasma. In patients treated with a potent HAART combination, the viral load rebound in CSF was always associated with a concomitant and more marked rebound in plasma. The present study and other longitudinal studies provide evidence that, during HAART, the virus may evolve independently in the CSF. The ultimate implications of these findings, e.g., on the development of CNS disease or progression of systemic disease, are still unknown.

Patients and methods

Patients from the present study belong to a larger group of patients with CNS complications admitted at the Clinic of Infectious Diseases of San Raffaele Hospital, Milano, Italy. Patients were selected for having a CSF sample taken both before starting HAART and during treatment, but after at least 9 months from baseline. This interval of $\geq$9 months was chosen considering that, during the first months of HAART, the virological response in CSF may be slower than in plasma and therefore detection of CSF virus in these cases may be consistent with a response, although slow, in this compartment. HAART consisted of a combination of at least three antiretroviral drugs, including NRTIs, non-nucleoside RTIs, and protease inhibitors. During the follow-up period, suboptimal combinations, e.g., two NRTIs or two protease inhibitors alone, were occasionally administered.

Baseline CSF and plasma samples were drawn for diagnostic purposes and after having obtained patient informed consent. CSF and plasma samples were obtained simultaneously, with the exception of patient 11 (Table 1) in whom plasma sampling preceded the lumbar puncture of 1 month. Sixteen of 18 patients had neurological complications at baseline, consisting of progressive multifocal leukoencephalopathy ($n=$ 5), toxoplasmosis ($n=$ 3), primary CNS lymphoma ($n=2$), cytomegalovirus encephalitis ($n=2$), ADC ($n=1$), herpes simplex virus type 1 encephalitis ($n=$ 1), or neurological abnormalities of unclear origin ($n=2$). In the two asymptomatic patients, CSF was drawn at the time of the staging of a non-Hodgkin lymphoma. Follow-up samples were drawn in concomitance with a new neurological episode (6 patients) or to further investigate persistent clinical or CSF abnormalities (12 patients).

HIV-1 levels were measured in both CSF and plasma by the Cobas Amplicor assay (Roche, Basel, Switzerland). Either the standard or the ultrasensitive version was used; however, the lower detection limit of HIV-1 RNA in this study was set at 400 copies/ml for all the cases. Resistance mutations were assesses by direct CSF and plasma sequencing of the RT and protease genes, as previously described (Cinque *et al*, 2001).

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