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Etravirine Concentrations in Cerebrospinal Fluid in HIV-Infected Patients

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Abstract

Objectives: To determine etravirine (ETR) concentrations in cerebrospinal fluid (CSF) in HIV-infected patients.

Methods: Twelve HIV-1 adult antiretroviral-experienced patients receiving an ETR-containing regimen for at least 1 month were enrolled. Both CSF and blood samples were taken around 12 h after the last ETR dose. LC/MS/MS was used to determine ETR concentrations, and HIV-1 viral load was determined by real-time PCR, (LOD, 40 copies/mL).

Results: Twelve blood and 12 CSF samples were collected. Median CD4 count was 333 (84-765) cells/uL and median HIV-1 viral load was <40 (<40-1777) copies/mL. Median time on ETR was 42.4 weeks (4-140).

Median ETR concentration in plasma was 611.5 (148-991) ng/mL. Median CSF ETR concentration was 7.24 (3.5-17.9) ng/ml; in all cases, values were above the IC50 range (0.39-2.4ng/ml). Median ETR CSF:plasma ratio was 0.01 (0.005-0.02). CSF viral load was > 40 copies/mL in one patient and plasma VL was still detectable after only 4 weeks of therapy.

Conclusions: ETR achieves concentrations several times above the IC50 range in CSF. All patients with undetectable plasma viral load were virologically suppressed in CSF while receiving an ETR-containing regimen. ETR may help in controlling HIV-1 in CNS.

Introduction

Neurocognitive impairment seems to be a frequent event in HIV-1-infected patients despite the advent of cART[1]. Patients with symptomatic HIV encephalopathy have increased viral replication within the central nervous system (CNS), but apparently even low-level viral replication in this reservoir while on combined antiretroviral therapy can promote local immune activation, an inflammatory response, and subsequent brain damage.[1,2] Moreover, the CNS may act as a separate viral reservoir, where HIV-1 strains with a different resistance pattern to that of plasma HIV-1 strains may be found.[3] Good penetration of antiretroviral (ARV) drugs into the cerebrospinal fluid, a suggested surrogate CNS marker, has been associated with a decrease in viral replication and an improvement in neurocognitive alterations assessed by neuropsychological tests[3]. Cerebrospinal fluid (CSF) penetration can vary between the different ARV compounds. Most nucleoside analogues (with the exception of tenofovir) and the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine penetrate well[4], as shown in previous studies. Efavirenz concentrations in the CSF are only 0.5% of plasma concentrations but exceed the wild-type IC(50) in nearly all individuals [5], whereas penetration is variable in the case of protease inhibitors. Indinavir achieves good concentrations, and darunavir and lopinavir fair concentrations, while atazanavir is associated with lower concentrations, although it has been suggested that IP levels would suffice to inhibit viral replication in some cases.

New ARV drugs approved over the last few years have led to the design of effective and well-tolerated regimens for naïve and experienced patients. RGV (raltegravir) exceeded the IC95 levels reported to inhibit wild-type strains in a proportion of studied samples while darunavir concentrations were detectable in all assessed samples; most of the

latter exceeded or were in the same range as levels needed to inhibit replication of the wild-type virus[6]. Finally, maraviroc CSF concentrations were several times above the median IC90 in two groups of HIV-infected patients[7].

Data on the distribution of etravirine (ETR) into human compartments other than plasma has not been published to date, but extremely high protein binding means extensive penetration is not to be expected. Interestingly, the addition of ETR to a tenofovir/abacavir/saquinavir/lopinavir/ritonavir regimen was associated with an improvement in a patient with acute HIV meningoencephalitis[8].

Patients and Methods

Twelve asymptomatic HIV-infected ARV pre-treated adult patients were enrolled in our HIV outpatient unit. In five of these patients, CSF maraviroc concentrations were also assessed[8]. All subjects had been taking ETR for at least 4 weeks as part of an ARV regimen. ETR dosage was set according to recommendations in the European Medicines Agency (EMA) scientific report[9]. Blood samples were obtained from each patient by peripheral venous puncture and CSF samples by lumbar puncture. All samples were centrifuged and frozen at -70ºC until analysis. Both blood and CSF samples were taken approximately 12 hours after the previous ETR dose, in order to obtain the lowest ETR concentration.

The study was approved by the hospital ethics committee and the Spanish Drug Agency, and patients gave written informed consent to participate.

Total ETR concentrations in plasma and CSF samples were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS), using positive turbo ion spray mode (Tandem Labs-New Jersey, 115 Silvia Street West Trenton, NJ 08628). The ion transition monitored was 514.1/389.1. The reverse-phase chromatography calibration

range was 0.250-1000 ng/mL in Li heparin plasma samples. The extraction procedure is based on protein precipitation with acetonitrile, using 50 µL of plasma. The internal standard was the stable isotope label for ETR.

HIV-1 viral load was quantified with a real-time PCR technique (Abbot Molecular Inc, Des Plaines, IL), performed according to the manufacturer's recommendations. The limit of detection of the method was 40 copies/mL.

Results

Eleven patients (91%) were men, and the mean age was 49.9 years. Median HIV-1 viral load was <40 (<40-1777) copies/mL and median CD4 count was 333 (84-765) cells/uL. Patients were receiving ETR for a median period of 34 (4-140) weeks. The pharmacokinetic and virologic data, and the ARV regimens received, are summarized in Table 1. Raltegravir (RGV) was given in 66% of cases, darunavir (DRV) in 58%, and maraviroc (MVC) in 41%. Nucleosides/tides were present in less than half of patients. ETR dosage was 400 mg/day and two patients were taking this dosage once a day. Median time period post dose was 12.5 hs (3 -16 hs).

In all samples, ETR concentrations exceeded the IC50 range (0.39-2.4ng/ml). Median ETR plasma concentration was 661.5 (148-991) ng/ml and median ETR CSF concentration was 7.24 (3.5-17.9) ng/ml. The median ETR CSF:plasma ratio was 0.01 (0.005-0.02) and the ETR CSF:estimated free plasma concentration (0.1% of the total ETR concentration) ratio was 12.06.

Plasma HIV-1 viral load was detectable (192 and 1777 copies/ml) only in two patients, one of whom had CSF VL below 40 copies/ml while the other had 111 copies/ml. These two patients had been receiving 4-drug ETV-containing regimens for 16 and 4 weeks, respectively. They had shown a very good initial virological response (viral load

reduction of 1.9-2.8 log), but still had detectable plasma HIV-1 RNA levels at the time of the study.

Discussion

As expected, ETR plasma concentrations in all patients were several times higher than the IC50 range. Moreover, ETR CSF concentrations were above this value in all patients. It should be taken into account that almost all the drug in CSF is protein unbound and therefore active. Thus, the minimal drug concentration needed to inhibit 50% of the virus in CSF would likely be even lower. Furthermore, the use of triple ARV therapy with CSF-penetrating drugs would assure sufficient antiviral activity in this reservoir.

This data is in accordance with results from a study recently presented by Best et al. at an international meeting which showed that median ETR concentration in 9 patients was 9.5 ng/ml (6.4-26.4)[10]. However, in that study plasma and CSF samples were taken at a median of 4.8 hs (2.9-5.7) and 4.9 hs (2.6-5.5) after the last ETR dose, respectively. All samples were therefore taken near the ETR Cmax, making it difficult to know whether levels would remain higher than the IC50 until the next dose of the drug. ETR is extensively bound (99.9%) to albumin and alpha1-acid glycoprotein, suggesting a poor presence in CNS. However, the fact that ETR CSF concentrations were several times higher than the protein-unbound percentage of plasma concentrations suggests that ETR penetrates the Blood Brain Barrier (BBB) mainly by transcellular diffusion due to its lipid solubility.

Interestingly, p-glycoprotein expressed in BBB is inhibited by ETR, but a clinically significant reduction of its activity would be achieved only with much higher ETR concentrations than those expected in human plasma. On the other hand, ETR is not a substrate of P-glycoprotein efflux pump.

Our observations provide evidence suggesting a role for ETR in the treatment of HIV-1 in the CNS. Moreover, the fact that nine out of the twelve patients were receiving a nucleoside-sparing regimen (n:6) or tenofovir (a drug with a known low CNS penetration) as the only nucleotide (n:3) reinforces the likelihood that sufficient antiviral activity can be generated in this compartment by combining drugs with at least some CSF penetration.

Knowledge is still very limited in this field, however, and many unanswered questions remain, such as the clinical significance of drug penetration into CSF and the number of penetrating drugs needed to reach viral suppression.

It has been suggested that even low-level CSF viral replication of under 40 copies/mL may be associated with continuous immune activation and subsequent brain damage.[1] However, despite these controversial data, the available evidence suggests that good penetration of ARV drugs into the CNS may help to prevent and improve neurocognitive disturbances in patients with a chronic disease, as well as i**nhibiting]** viral dissemination into the CNS in the initial phases of infection.[1]

Conclusion

Our data suggest that ETR may contribute to inhibit HIV-1 replication in CNS and that combined nucleoside-sparing regimens, including new ARV drugs, seem to be locally active.

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N	PlasmaVL (copies/ml)	CSF VL (copies/ml)	CD4 (cel/ul)	Weeks on ETR	PlasmaConc (ng/mL	CSFConc (ng/mL	CSF:Plasma Ratio	Concomitant ARV Treatment
2	<40	<40	221	5	991	7,58	0,007	RGV-MVC-DRVr
3	1777	111	120	4	666	6,99	0,01	RGV-MVC-DRVr
4	<40	<40	231	58	281	3,67	0,01	RGV-MVC-DRVr
5	<40	<40	384	50	277	8,24	0,03	RGV-MVC-DRVr
9	<40	<40	486	140	148	3,82	0,02	RGV-DRVr
1	<40	<40	759	24	589	5,89	0,01	RGV-MVC
6	192	<40	330	16	421	7,05	0,016	RGV-DRVr-TDF*
8	<40	<40	84	92	705	17,9	0,02	RGV-TDF
12	<40	<40	153	12	697	8,33	0,01	DRVr-TDF
7	<40	<40	459	44	669	16,4	0,02	TDF/FTC
10	<40	<40	336	52	531	7,43	0,01	AZT-ddI
11	<40	<40	765	12	634	3,59	0,005	ABC-3TC*
	Median: <40	**Median: <40**	**Median: 333**	**Median: 34**	**Median: 611,5**	**Median: 7,24**	**Median: 0,01**	

Table 1. Pharmacokinetic, virological and therapeutic data of enrolled patients

Abbreviations: CSF, cerebrospinal fluid; VL, viral load; Conc., concentration; ARV, antiretroviral;ddI, didanosine; ETR, etravirine; DRVr, darunavir/ritonavir; TDF, tenofovir; AZT, zidovudine; ABC, abacavir; FTC, emtricitabine; 3TC lamivudine, MVC, maraviroc; RGV, raltegravir.

* Patient receiving ETR once a day.

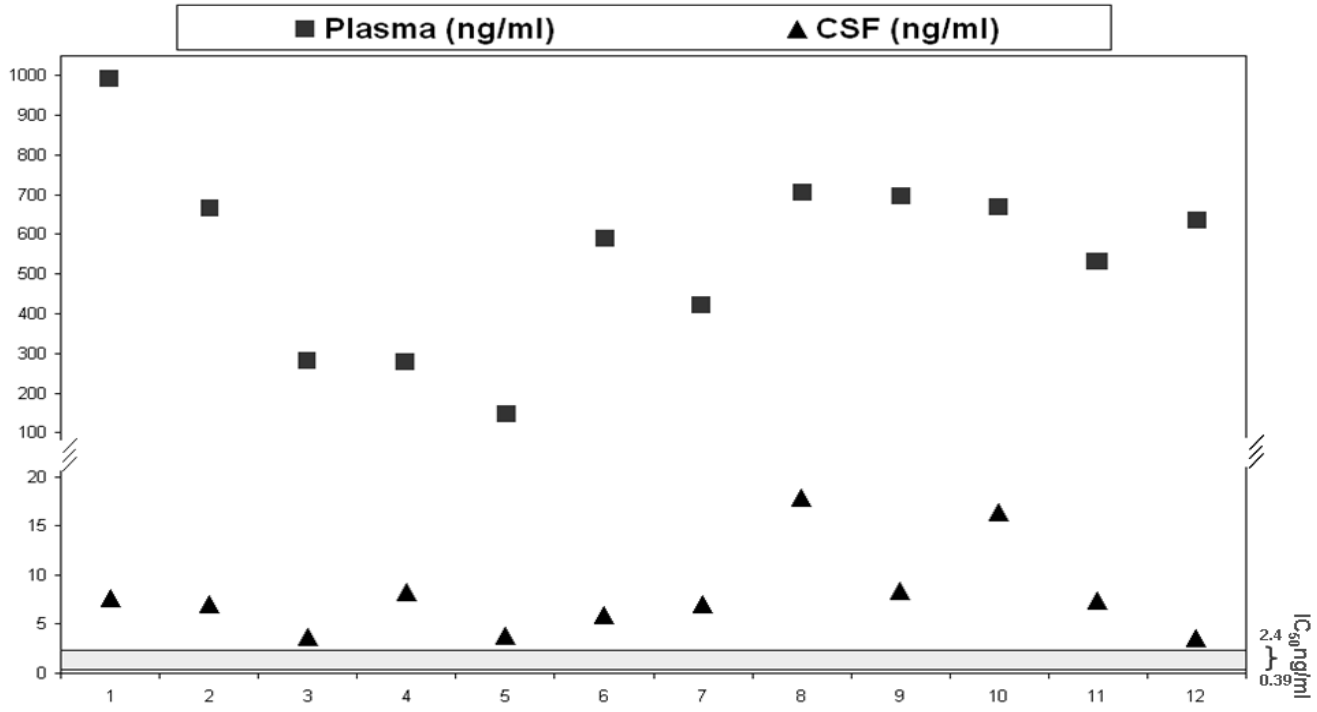

Figure 1. Plasma and CSF concentrations of etravirine