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Hepatitis B virus compartmentalization in the cerebrospinal fluid of HIV-infected patients

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Abstract

We detected hepatitis B virus (HBV) DNA in the cerebrospinal fluid (CSF) of 26 adolescents co-infected with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) with neurological disease and studied compartmentalization of HBV in the CSF. More than half of the subjects with positive HBV DNA plasma also had CSF positive for HBV. CSF HBV DNA was found in subjects with preserved blood–brain barrier integrity. In a subgroup of these subjects, compartmentalized evolution of HBV was demonstrated by distinct profiles of resistance mutations. Future studies are warranted to determine the clinical significance of HBV presence in the CSF and its contribution to HIV-associated neurological disease.

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Transparency declaration
The authors declare that they have no conflicts of interest.

Authorship/Contribution
DD designed the study; LE collected the data and drafted the manuscript; GT, SR and SM performed the laboratory tests; DD, LE, SR, DS, SM, SL and CA interpreted the data; DS, SM, SR and CA coordinated writing the manuscript; and all authors reviewed the manuscript and approved the version for publication.

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Keywords

Cerebrospinal fluid; co-infection; compartmentalization; hepatitis B virus; human immunodeficiency virus

Introduction

Non-hepatic reservoirs of hepatitis B virus (HBV) have been demonstrated in neuronal cells [1]. Unique nucleotide substitutions were found in the HBV sequences from the cerebrospinal fluid of a patient with transverse myelitis [2]. Few data exist on the impact of HBV in the central nervous system (CNS) of human immunodeficiency virus (HIV) - infected individuals. Romania has a highly homogeneous cohort of HIV-infected patients infected parenterally during their first years of life (1987–1990), with HIV clade F1. A substantial percentage (78%) of this cohort has serological evidence of past or present HBV infection [3], possibly acquired concomitantly with HIV infection. We aimed to evaluate the presence and genotypic characteristics of HBV DNA in the cerebrospinal fluid (CSF) in a group of patients from this cohort.

Materials and methods

We retrospectively analysed all available stored paired blood plasma and CSF samples (between 2003 and 2009) from HIV-1-infected children with a positive plasma hepatitis B surface antigen who were undergoing a lumbar puncture for diagnosis of CNS disorders. HBV viral loads were measured using the Cobas Amplicor HBV Monitor Test (Roche Diagnostics, Mannheim, Germany), with a linear range of 60–38000 IU/mL. HIV-1 viral loads were determined using Cobas Amplicor HIV-1 Monitor v1.5 (Roche Diagnostics), with a linear range of 50–100000 copies/mL (ultrasensitive method), and 400–750 000 copies/mL (standard method). The integrity of the blood–brain barrier was evaluated using a CSF/serum albumin index [4], with normal value <9.

In a subgroup of five patients with sufficient volume of stored paired CSF–plasma samples and an HBV viral load of >1000 IU/mL, the presence of antiviral-resistant variants was analysed using INNO-LiPA HBV DR v2 (Innogenetics NV, Ghent, Belgium), a reverse hybridization line probe assay, that can detect genetic variants and minority virus populations not evidenced by sequencing [5]. In one patient we performed population-based sequencing of HBV DNA from blood and CSF (Abbot, North Chicago, IL, USA).

Results

The study group consisted of 26 individuals (15 males) infected parenterally (Table 1). All had evidence of chronic hepatitis B without cirrhosis or hepatic insufficiency and alanine amino-transferase values less than two times the upper limit of the normal range. None of the participants had histological evaluation for liver fibrosis. HBV DNA was quantified in all 26 participants: 18 had detectable levels in the blood (mean 6.01 ± 2.09 log10 IU/mL) and 11 in the CSF also (mean 4.06 ± 1.06 log10 IU/mL) (Table 2). Viral loads were significantly different between compartments ($p < 0.0001$), but positively correlated ($r =$

0.82, p < 0.0001, 95% CI for r = 0.64 to 0.92). Additionally, there was a positive correlation between HBV DNA and HIV RNA copy number in plasma and CSF (plasma *r* = 0.59, p 0.001; CSF r = 0.49, p 0.01). None of the eight patients with undetectable plasma HBV DNA had detectable CSF HBV DNA.

Twenty participants were taking combination antiretroviral therapy (cART) at the time of lumbar puncture and 18 had received cART regimens containing lamivudine. None of the participants had previous or current use of other anti-HBV nucleotide/nucleoside analogues. Out of 15 participants currently exposed to lamivudine, 12 patients had undetectable CSF HBV DNA. Five of the 15 patients were failing their cART regimen, but only two of them had detectable HBV DNA.

In five participants with detectable HBV DNA in blood and CSF (four of them with previous exposure to lamivudine), HBV genotypic analysis for drug resistance was performed. Two participants demonstrated concordant profiles in blood and CSF. The other three participants demonstrated HBV genotypic resistance and genotypic discordance between blood and CSF. Two had the primary resistance mutation M204V and the compensatory L180M mutation, in both the blood and CSF, while having the V173L mutation only in plasma and rtA181T mutation only detectable in CSF. One participant had the HBV mutations L180M, M204V and A181T only detectable in CSF and for the patient in whom we performed a population-based sequencing of HBV DNA isolated from blood and CSF there were no differences between the sequences.

Discussion

This is the first report to document the presence of HBV DNA in the CSF of HIV/HBV co-infected patients and to provide evidence of HBV compartmentalization between blood and CSF. These findings may be important in understanding how HBV affects the CNS, in the setting of HIV co-infection. A strong association between HIV presence and expression of DNA viruses, including HBV, was shown *in vitro* [6]. Accordingly, in our study all subjects with HBV DNA in the CSF also had detectable CSF HIV RNA.

Although not all patients with detectable plasma HBV DNA also had detectable viral load in the CSF, in several subjects there was evidence of CNS compartmentalization through genotypic analysis of HBV drug resistance mutations as confirmed by a previous report [2]. This suggests that HBV can replicate independently in the CNS. which may function as a sanctuary during the development of HBV drug resistance similar to what has been demonstrated for HIV [7]. Interestingly, most of the subjects with current exposure to lamivudine had undetectable HBV in the CSF, although it is known that prolonged lamivudine therapy induces emergence of HBV-resistant variants at a higher rate in HIV–HBV co-infected patients than in HBV mono-infected ones [8].

Although informative, our observational study has several limitations: the small number of subjects and absence of mono-infected control groups limited the power to determine the clinical significance of the presence or level of HBV in the CSF for neurological

dysfunction, absence of longitudinal analyses, and clonal sequence analysis of HIV or HBV on the paired samples.

In summary, we show presence of HBV DNA in the CSF in a number of HIV/HBV co-infected individuals, and using genotypic analysis we demonstrate that there is compartmentalization of HBV between the CSF and plasma. Nonetheless, it is still unclear if presence of HBV DNA in the CSF is associated with neurological complications or if antiretroviral regimens containing lamivudine may benefit the HIV/HBV co-infected patients with neurological disease but we believe that this is a promising lead to pursue.

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TABLE 1

Demographic, clinical and laboratory characteristics of the HBsAg-positive patients with paired cerebrospinal fluid–plasma samples

No. of patients		**26**
Gender	M/F	15/11
Age at diagnosis of HIV-infection (years)	Median (limits)	10 (1–20)
Age at diagnosis of neurological complication (years)	Mean ± SD	17.0 ± 2.12
HIV-1 transmission route	Parenteral (non-IDU)	25
	Vertical	1
CD4 count (lf/mm^3)	Median (range)	164 (1–738)
Mean HIV RNA ± SD (log_{10} copies/mL)	Plasma	3.86 ± 1.40
	CSF	3.07 ± 1.18
History of exposure to ART		20
History of exposure to 3TC		18
On ART containing 3TC before the neurological complication		15
HBV markers	HBeAg positive	13 of 22 tested
	HDV antibodies positive	4 of 20 tested
	HBV DNA plasma (IU/mL) mean ± SD	4.7 ± 2.63***
	HBV DNA CSF (IU/mL) mean ± SD	2.74 ± 1.33***
Positive HBV DNA (>60 IU/mL)	Plasma	18 patients
	CSF	11 patients
HBV DNA log_{10} IU/mL in patients exposed to 3TC before lumbar puncture (n = 15)	Mean Plasma values	3.57 ± 2.10**
	Mean CSF values	2.16 ± 0.92*
HBV DNA log_{10} IU/mL in patients not exposed to 3TC before lumbar puncture (n = 11)	Mean Plasma values	6.26 ± 2.55**
	Mean CSF values	3.54 ± 1.42*

* $p < 0.01$;

** p 0,007;

*** p 0.0001.

Abbreviations: 3TC, lamivudine; ART, antiretroviral treatment; CSF, cerebrospinal fluid; HbeAg, hepatitis B e antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; IDU, intravenous drug user.

TABLE 2

Patient immunological, serological and virological profiles.

Patient ID#	Gender	Age (years)	CD4 (cells/mL)	Hbe Ag	HDV Ab	HBV DNA plasma log_{10} IU/mL	HBV DNA CSF log_{10} IU/mL	HIV RNA plasma log_{10} c/mL	HIV RNA CSF log_{10} c/mL	Albumin index ($N < 9$)	CSF albumin levels (g/L)	CSF pleocytosis (WBC/mL of CSF)	Previous cART	Length of current exposure to 3TC (months)	Neurological diagnosis	MRI demyelinating lesions
1	M	21	7	N/A	N/A	9.46	4.7	4.61	4.3	10.81	0.4	4	no	—	HIVE	yes
2	F	16	125	pos	N/A	8.84	3.36	6.01	2.48	N/A	N/A	N/A	yes	—	stroke	yes
3	M	18	37	N/A	N/A	8.72	4.69	2.79	2.6	18.86	0.66	46	yes	5	stroke	yes
4	M	17	490	pos	N/A	8.24	5.58	5.11	5.85	27.73	0.99	5	yes	—	HIVE	yes
5	M	17	220	N/A	N/A	7.65	5.36	4.18	3.44	17.55	0.66	N/A	no	—	viral encephalitis	no
6	F	15	8	N/A	N/A	7.54	3.42	4.58	2.72	2.62	0.1	3	no	—	viral encephalitis	yes
7	F	20	59	pos	neg	6.87	4.12	5.92	5.78	8.88	0.3	10	yes	2	CMV encephalitis	yes
8	M	14	13	pos	neg	6.34	3.3	5.34	4.36	12.35	0.4	5	No	—	Tx	—
9	M	17	1	pos	neg	5.77	4.91	5.61	2.89	73.33	1.98	20	yes	—	SME	yes
10	M	15	182	pos	neg	5.73	2.98	5.15	2.59	15.31	0.66	1	yes	—	facial paresis	normal
11	F	20	348	pos	N/A	5.73	1.78	4.11	3.32	6.01	0.2	3	no	—	HIVE	—
12	M	16	380	pos	N/A	5.07	1.78	2.25	2.25	2.06	0.1	3	yes	60	stroke	—
13	M	14	19	pos	neg	4.85	1.78	5.82	3.69	2.78	0.1	1	yes	49	PML	yes
14	F	20	231	neg	N/A	4.6	2.26	3.6	2.6	2.49	0.1	4	yes	13	SME	yes
15	M	15	7	pos	N/A	3.38	1.78	2.6	2.6	16.92	0.66	3	yes	1	PML	yes
16	M	16	306	pos	pos	3.24	1.78	2.6	2.6	2.65	0.1	2	yes	9	behaviour changes	no
17	M	17	16	neg	pos	3.15	1.78	4.8	2.6	2.53	0.1	1	yes	29	SME	yes
18	F	18	325	neg	neg	3.03	1.78	3.79	2.25	18.33	0.66	6	yes	36	epilepsy	normal
19	F	16	8	pos	pos	1.78	1.78	3.2	2.6	2.90	0.1	3	yes	45	evaluation Hodgkin lymphoma	normal
20	F	16	305	neg	N/A	1.78	1.78	3.62	4	33.08	1.32	22	yes	viral encephalitis	—	
21	M	16	1	neg	pos	1.78	1.78	2.25	2.43	3.65	0.1	3	yes	42	SME	yes
22	F	18	220	neg	neg	1.78	1.78	1.7	1.7	2.54	0.1	12	yes	22	HIVE	no
23	M	18	738	N/A	N/A	1.78	1.78	1.7	1.7	2.45	0.1	2	yes	69	HIVE	—
24	F	20	406	neg	neg	1.78	1.78	1.67	1.67	2.34	0.1	2	yes	24	HIVE	—
25	F	19	255	neg	neg	1.78	1.78	2.72	1.7	2.38	0.1	4	yes	9	HIVE	—
26	M	21	146	neg	neg	1.78	1.78	4.8	5.1	6.90	0.2	22	no	—	HIVE	yes

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Abbreviations: HBe Ag, hepatitis B e antigen; HDV Ab, hepatitis D virus antibodies; HBV, hepatitis B virus; CSF, cerebrospinal fluid; WBC, white blood cells; cART, combination antiretroviral therapy; HIVE, HIV encephalopathy; SME, measles inclusion body encephalitis or subacute myoclonic measles encephalitis; PML, progressive multifocal leucoencephalopathy; Tx, cerebral toxoplasmosis.