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CASE REPORT

Raltegravir resistance in the cerebrospinal fluid

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Abstract We report the first published case of integrase inhibitor resistance in the central nervous system compartment in the absence of evidence of integrase inhibitor resistance in the plasma of a patient without human immunodeficiency virus (HIV)-encephalitis in the context of other HIV-associated central nervous system infections.

Keywords Raltegravir · Integrase inhibitor · Resistance · Cerebrospinal fluid · Central nervous system

Introduction

Discordant human immunodeficiency virus (HIV) RNA levels between the cerebrospinal fluid (CSF) and plasma have been described in up to 13 % of patients with suppressed plasma viral loads on differing antiretroviral (ARV) regimens [1]. This discordance between the plasma and central nervous system (CNS) compartments may be more frequently observed in subjects with HIV-related CNS disease [1]. Differences in ARV drug resistance mutations between plasma and CNS compartments have also been reported [2].

Case report

We report the case of a 49-year-old Korean man diagnosed with HIV infection in 1998 in Sydney, Australia. After his initial HIV diagnosis, he did not attend the routine follow-up programme until 2005 when he presented with secondary syphilis, which was treated in the community with three intramuscular injections of benzathine penicillin 1 week apart. In April 2007 he presented to HIV services with *Pneumocystis jirovecii* pneumonia (PCP), cutaneous Kaposi's sarcoma and a mild sensory neuropathy, at which time his CD4+ cell count was 39 cells/μL (3 %) and the HIV viral load was 384,900 copies/mL. PCP was treated with 21 days of co-trimoxazole and a short course of prednisone. Three weeks after discharge from hospital, he presented with multidermatomal shingles (affecting the left T5 and T6 dermatomes) and a severe headache. CSF examination revealed an elevated protein level (2.69 g/L), and varicella-zoster virus (VZV) DNA was detected by PCR. Testing for other viruses and organisms was negative. He was treated with high-dose intravenous aciclovir (10 mg/kg 8 hourly) for 2 weeks. Genotypic resistance testing [reverse transcriptase (RT) and protease (PR) assays] was performed and revealed wild-type virus. The patient was initiated on a regimen of abacavir 600 mg, lamivudine 300 mg, and atazanavir 300 mg/ritonavir 100 mg, all once daily.

Despite a reasonable immunological response to ARV over the next 2 years with a rise in the CD4 count to 416 cells/μL (16 %), the patient did not achieve an undetectable plasma viral load in this period. In September

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2009, repeated genotypic resistance testing (RT assay) revealed the M184V mutation (plasma HIV RNA 420 copies/mL). Due to concerns regarding his unsuppressed HIV viral load, drug resistance mutation, persistent neuropathic symptoms and desire to be on a once-daily regimen, the patient was switched to raltegravir 800 mg, darunavir 900 mg, and ritonavir 100 mg as a daily regimen in November 2010.

In February 2011, the patient achieved an undetectable plasma HIV RNA load, but developed headaches, fever and meningismus. CSF examination at this time revealed a white blood cell count (WBC) of 1,100 cells/μL, an elevated protein level (5.25 g/L), a CSF HIV-1 RNA load of 460 copies/mL, neopterin at 302 nmol/L and again a positive VZV DNA by the PCR assay (Table 1). He was therefore treated for VZV meningitis with high-dose intravenous aciclovir (10 mg/kg 8 hourly) for 2 weeks. The patient improved clinically, but in July 2011 he developed bilateral pain and sensory disturbances around the T9–T11 dermatome region. A spinal magnetic resonance imaging (MRI) scan demonstrated a single hyperintense lesion in the T2 region of the anterior cord considered to be compatible with VZV myelitis, and the patient was treated with a further 2-week course of high-dose intravenous aciclovir. Following this, repeat CSF examination showed improvement in the total protein (1.40 g/L) and WBC count (30 cells/μL) with a negative VZV DNA by PCR. CSF HIV RNA remained detectable at 400 copies/mL despite full suppression in the plasma (Table 1). There were no overt symptoms of neurocognitive impairment, and he was maintained on the same ARV regimen with secondary prophylaxis for VZV disease with valaciclovir 1 g twice daily.

In December 2011, the patient moved to London. On presentation he continued to describe headaches and a

Table 1 Chronological findings from relevant cerebrospinal fluid and plasma examinations

Dates	Plasma HIV-1 RNA (copies/mL)	Plasma CD4+ cell count, cells/μL (%)	CSF HIV-1 RNA (copies/mL)	CSF Protein (g/L)	CSF neopterin (nmol/L)[a]	CSF glucose (mmol/L)	CSF VZV DNA PCR	Syphilis serology[b]
1 May 2007: plasma sample	384,900	39 (3)	–	–	–	–	–	TPPA++, RPR negative
No resistance mutations in RT or PR assay; no IN sequence assay performed								
24 May 2007: CSF sample	–	–	–	2.687	160	2.6	Detected	–
2 September 2009: plasma sample	420	416 (16)	–	–	–	–	–	TPPA++, RPR negative
M184V resistance mutation in RT assay; no IN sequence assay performed								
November 2010	cART switched from 3TC/ABC/ATV/r to RAL/DRV/r (od)							
1 February 2011: CSF sample	–	–	460	5.245	302	2.9	Detected	–
9 February 2011: CSF sample	–	–	200	4.405	160	4.4	Detected	–
12 August 2011 CSF sample	–	–	400	1.427	87		Not detected	–
9 December 2011: plasma sample	<50	520 (33)	–	–	–	–	–	TPPA++, RPR+ (1/8)
28 December 2011: CSF sample	–	–	1,438	1.480	–	3.5	–	TPPA++, RPR negative
R5 tropic virus predicted. L74V and M184V detected in RT assay; N155H in IN assay								
27 January 2012: plasma sample	–	–	–	–	–	–		TPPA++, RPR+ (1/4)
27 January 2012: CSF sample	–	–	–	1.390	–	3.8	–	TPPA++, RPR negative
February 2012: plasma sample	<50	520 (26)	–	–	–	–	–	TPPA++, RPR+ (1/2)
X4 tropic virus predicted (test performed on a proviral DNA as virus load <50)								

HIV Human immunodeficiency virus, *CSF* cerebrospinal fluid, *VZV* Varicella Zoster Virus, *RT* reverse transcriptase, *PR* protease, *IN* integrase, *cART* combination antiretroviral therapy, *3TC* lamivudine, *ABC* abacavir, *ATV* atazanavir, *r* ritonavir, *RAL* raltegravir, *DRV/r* darunavir

[a] Normal range: 0–13 nmol/L

[b] *TTPA Treponema pallidum* particle agglutination assay, *RPR* Rapid Plasma Reagin, – not determined

cerebral MRI scan showed a non-specific, oval-shaped lesion in the region of the left temporal white matter (Fig. 1) and a small area of abnormal enhancement in the ventral part of the cord at the T2 level. The patient tested positive for serum syphilis serology on the *Treponema pallidum* particle agglutination assay (TPPA) and had a rapid plasma reagin (RPR) titre of 1:8. A repeat CSF examination could not rule out the possibility of neurosyphilis based on a positive TPPA titre, negative RPR titre and a raised protein concentration of 1.48 g/L. Initially the patient declined recommended therapy (17 days of either intravenous penicillin or high-dose oral penicillin therapy) and therefore received three doses of intramuscular benzathine penicillin 2.4 MU 1 week apart. At this time, it was noted that the CSF HIV-1 RNA load remained detectable at 1,438 copies/mL despite full suppression in plasma (Table 1). Genotypic resistance testing of CSF HIV-RNA revealed the L74V and M184V mutations (RT assay) and the N155H mutation in integrase. Viral tropism was inferred based on the results of genotypic analysis of the HIV-1 gp120 V3 loop region from CSF. Edited sequences were submitted to the geno2pheno co-receptor predictive algorithm [3]. CCR5-tropic virus was predicted in the CSF with a false positive rate (FPR) of 72.7 % using a cut-off of 5.75 %. A sample performed using proviral DNA from peripheral blood lymphocytes 6 weeks later predicted a CXCR4-tropic virus (FPR 1.7 % using a cut-off of 15 %). In view of the patient's symptomatology and the CSF genotypes determined, his ARV regimen was intensified with maraviroc 300 mg once daily and zidovudine 300 mg twice daily.

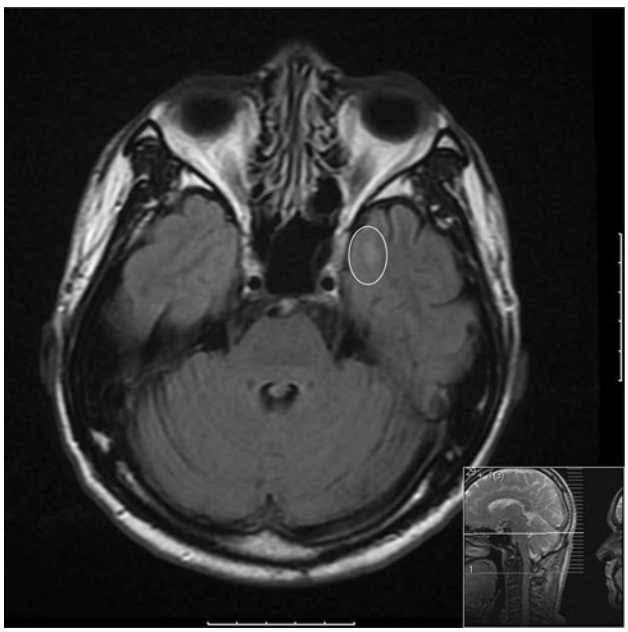

Fig. 1 Magnetic resonance imaging scan of the brain. FLAIR sequence. A non-specific *oval-shaped* lesion is seen in the left temporal white matter

In January 2012, due to ongoing headaches and low plasma RPR titres of 1:4, a further lumbar puncture was undertaken, revealing an elevated CSF protein concentration (1.39 g/L), a positive TPPA titre and a negative RPR. The patient subsequently agreed to complete optimal therapy for neurosyphilis with amoxicillin 2 g three times daily and probenecid 500 mg four times daily for 21 days. Following intensification of antiretroviral therapy and completion of therapy for neurosyphilis, the headaches resolved and the patient felt well with a continued undetectable plasma HIV RNA load despite withdrawal of the zidovudine component after 1 month for intractable nausea. A follow-up CSF examination to assess HIV RNA and ARV drug levels in the CSF and a repeat MRI scan of the brain are planned.

Discussion

To our knowledge, this is the first reported case of integrase inhibitor resistance in the CNS compartment despite no evidence of resistance in the plasma compartment in a patient without HIV-encephalitis [4]. Resistance to the HIV integrase inhibitor, raltegravir, is associated with treatment failure and involves mutations primarily at positions N155, Q148 and Y143 within the integrase gene [5].

Several clinical factors have been reported to be associated with discordant viral replication in the CNS and plasma compartments, including concomitant CNS infections [6, 7], previous virological failure in the plasma compartment [2], previous interruptions of treatment [8] and insufficient drug exposure in the CSF [1]. Many of these risk factors were present in this case.

Concurrent CNS infections, such as VZV or neurosyphilis [9], may lead to intrathecal immune activation, which has been reported to be associated with discordant HIV replication between the plasma and CNS compartment [10]. Indeed, the raised concentration of neopterin observed on serial CSF examinations is in keeping with this hypothesis [10].

Regarding the origin of the N155H mutation, high viral genetic diversity between the CSF and plasma in patients with HIV encephalitis has been described in association with undetectable plasma HIV RNA, which may be caused by independent replication and evolution of resistance within the CNS [11]. Although an integrase mutation in the CSF of our patient in the presence of undetectable plasma HIV RNA may suggest autonomous viral replication in the CNS, transfer of concordant mutations in plasma to the CNS cannot be completely ruled out in the absence of a plasma HIV genotypic resistance test. Of note, the presence

of the M184V resistance mutation in the CNS compartment without nucleoside reverse transcriptase inhibitor selective drug pressure at that time may reflect low viral turnover rate in the CNS in the presence of boosted darunavir, as indicated by the relatively low HIV viral load found in the CSF. In terms of tropism, HIV-1 found in the CNS compartment predominantly uses CCR5, and this tropism is usually concordant with HIV tropism in the plasma [12]. However, discordance has been described between viral tropism in the CSF and plasma and, as in our case, there may be clinical benefits of using maraviroc in such situations [12].

Additionally, discordance between CSF and plasma viral replication may be associated with inadequate drug exposure in the CNS due to pharmacokinetic reasons or poor compliance. The use of raltegravir twice daily has the potential to achieve local inhibitory concentrations for wild-type HIV in CSF, although inter-individual variability is reportedly high [13]. However, to our knowledge, no data exist for the CNS penetration of once-daily raltegravir, which may be associated with lower CSF exposure, especially at trough concentrations. Raltegravir CSF concentrations were not assessed in our patient.

Another explanation for the presence of integrase-resistant virus is the potential acquisition of a resistant HIV strain. While this is unlikely to have occurred at the time our patient initially acquired HIV infection, which was prior to the introduction of integrase inhibitors, a recent HIV superinfection is possible. Acquisition of a second strain of HIV can be associated with symptoms of seroconversion illness, which may explain the onset of his neurological symptoms; meanwhile, the suppressive effects of darunavir/ritonavir may have been sufficient to prevent overt viral rebound in plasma, making it unfeasible to determine resistance in the plasma compartment.

In conclusion, this is the first reported case of integrase inhibitor resistance in the CNS compartment despite no evidence of resistance in the plasma compartment of a patient without neurocognitive symptoms. A prompt CSF evaluation assessing HIV RNA level, viral resistance and tropism can be useful in individuals with any neurological symptoms despite undetectable plasma HIV RNA due to potential emergence of resistance within the CNS compartment which may occur with newer antiretroviral agents.

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Conflict of interest AW has received honoraria or research grants, or been a consultant or investigator, in clinical trials sponsored by Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen Cilag, Roche, Pfizer and ViiV Healthcare. All remaining authors declare no conflicts of interest.

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