HIV-2 viral tropism influences CD4+ T cell count regardless of viral load

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Received 30 August 2013; returned 2 October 2013; revised 13 March 2014; accepted 21 March 2014

Background: HIV-2 infection is characterized by low plasma viraemia and slower progression to AIDS in comparison with HIV-1 infection. However, antiretroviral therapy in patients with HIV-2 is less effective and often fails to provide optimal CD4 recovery.

Methods: We examined viral tropism in persons with HIV-2 infection enrolled in the HIV-2 Spanish cohort. Viral tropism was estimated based on V3 sequences obtained from plasma RNA and/or proviral DNA.

Results: From a total of 279 individuals with HIV-2 infection recorded in the Spanish national register, 58 V3 sequences belonging to 42 individuals were evaluated. X4 viruses were recognized in 14 patients (33%). Patients with X4 viruses had lower median CD4+ cell counts than patients with R5 viruses [130 (17–210) versus 359 (180–470) cells/mm³; P = 0.007]. This was true even considering only the subset of 19 patients on antiretroviral therapy [94 (16–147) versus 184 (43–368) cells/mm³; P = 0.041]. In multivariate analysis, significant differences in CD4+ cell counts between patients with X4 and R5 viruses remained after adjusting for age, gender, antiretroviral therapy and viral load.

Conclusions: The presence of X4-tropic viruses in HIV-2 infection is associated with low CD4+ cell counts, regardless of antiretroviral treatment. Along with CD4+ cell counts, viral tropism testing may assist decisions about when to initiate antiretroviral therapy in HIV-2-infected individuals.

Keywords: CCR5 antagonists, proviral load, maraviroc

Introduction

HIV-2 infection is endemic in certain areas of West Africa, where 5%–10% prevalence rates have been reported in some populations, but the virus has limited spread worldwide. Compared with HIV-1, HIV-2 infection results in lower viral loads, slower CD4 declines and slower progression to AIDS. However, HIV-2-infected individuals are at risk for developing opportunistic conditions once severe immunodeficiency develops. Given that antiretroviral drugs have mainly been optimized for the treatment of HIV-1 infection, therapies for HIV-2 are currently limited. HIV-2 is naturally resistant to non-nucleoside reverse transcriptase inhibitors and fusion inhibitors, and less susceptible to most protease inhibitors, such as fosamprenavir, atazanavir and tipranavir. Fortunately, clinical and in vitro studies show that most nucleos(t)ide reverse transcriptase inhibitors and integrase inhibitors exhibit good antiviral activity against HIV-2. Information on the susceptibility of HIV-2 to CCR5 coreceptor antagonists is important, since these agents may provide a desirable alternative option within the HIV-2 armamentarium.

Primary CCR5-tropic isolates of HIV-2 are inhibited in vitro by maraviroc. Because CCR5 antagonists only block infections produced by R5 viruses, it is mandatory to assess viral tropism before testing any susceptibility of HIV-2 to maraviroc. A recent report has identified four major genetic determinants of tropism in the V3 loop of the env gp105 of HIV-2. Furthermore, prediction of HIV-2 tropism using viral sequences obtained from proviral DNA collected from peripheral blood mononuclear cells (PBMCs) has shown to be accurate and therefore a suitable tool in HIV-2 patients with low or undetectable viral load.

Besides its therapeutic interest, viral tropism has been shown to influence disease progression in HIV-1 infection; in fact the presence of X4 viruses has been associated with low CD4+ cell counts, even when patients are receiving antiretroviral treatment.

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therapy. A similar effect might occur in HIV-2 infection. The aim of our study was to examine viral tropism in patients belonging to the HIV-2 Spanish cohort and determine its influence on disease progression.

Patients and methods

The Spanish HIV-2 national register is a publicly funded database that collects information from all individuals diagnosed with HIV-2 infection in Spain since 1989. A centralized repository of stored clinical samples, including PBMCs and plasma, functions in parallel and was used for the current study. The study was approved by the hospital ethics committee.

Plasma HIV-2 RNA was quantified using a non-commercial real-time PCR assay at Hospital Carlos III. The region amplified was the long terminal repeat region, for which the primers and probe have been described elsewhere. Both HIV-2 groups A and B are reliably detected with this assay.

Results are given as percentages and median values with IQRs.

Statistical analysis

Results are given as percentages and median values with IQRs. Comparisons were made using the χ²-test, with Fisher’s correction when appropriate or the Mann–Whitney U-test for the comparison of quantitative variables. Univariate and multivariate analyses were performed by logistic regression including gender, age, viral load, CD4+ cell count and antiretroviral therapy as variables. Differences were considered as significant only when P values were <0.05. All analyses were performed using SPSS software (version 15.0).

Results

From a total of 279 patients with HIV-2 recorded in the Spanish national register, 58 V3 sequences belonging to 42 distinct individuals were evaluated. Forty of these samples were drawn from plasma RNA and 18 from proviral DNA. Lack of plasma and/or PBMCs and low plasma viraemia or proviral DNA precluded obtaining results in the remaining subjects owing to unsuccessful amplification of the genetic material.

The median age of the study population at HIV-2 diagnosis was 44 years; 28 (67%) were male, 38 were infected with group A virus, 27 (64%) were immigrants from sub-Saharan Africa, 8 (19%) were native Spaniards, 4 (9.5%) were from Portugal and 1 was born in Costa Rica (the country of origin was not known for 2 patients). At the time of testing, the median plasma HIV-2 RNA was 3.28 (2.16–4.10) log copies/mL and the median CD4+ cell count was 227 (111–444) cells/µL. A total of 19 (45%) patients were on antiretroviral therapy at the time of viral tropism assessment. There were no significant differences in terms of age, gender, country of origin and HIV-2 viral subtype comparing the overall cohort (279 patients) and the patients selected for this analysis. Regarding viral load and CD4+ cell counts, the selected patients tended to have lower CD4+ cell counts (227 versus 315) and higher viral load values (3.28 versus 2.74) than the global population, although this did not reach statistical significance.

Overall, 28 (67%) individuals were estimated to be infected with R5 viruses whereas 14 (33%) harboured X4 HIV-2 strains. Any mutation at residue L18 was found in 12 patients, mutation V19K/R was present in 8 individuals, V3 global net charge >6 and/or insertions at position 24, 9

Table 1. Main characteristics of the HIV-2 study population according to viral tropism

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>R5</th>
<th>X4</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n (%)</td>
<td>42</td>
<td>28 (67)</td>
<td>14 (33)</td>
<td>—</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>28 (67)</td>
<td>17 (61)</td>
<td>11 (79)</td>
<td>0.73</td>
</tr>
<tr>
<td>Age (years), median (IQR)</td>
<td>44 (39–50)</td>
<td>43 (38–48)</td>
<td>45 (40–50)</td>
<td>0.73</td>
</tr>
<tr>
<td>HIV-2 RNA (log copies/mL), median (IQR)</td>
<td>3.3 (2.2–4.1)</td>
<td>3.1 (2.1–3.9)</td>
<td>3.8 (2.9–4.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>CD4 count (cells/µL/mm³), median (IQR)</td>
<td>227 (111–444)</td>
<td>359 (180–470)</td>
<td>130 (17–210)</td>
<td>0.007</td>
</tr>
<tr>
<td>On antiretroviral therapy, n (%)</td>
<td>19 (45)</td>
<td>11 (39)</td>
<td>8 (57)</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Discussion

This study has characterized viral tropism in a relatively large population of patients with HIV-2 living in Spain. Based on a previously validated genotypic tool, X4 viruses were present overall in one-third of the population, a rate somewhat comparable to that reported in HIV-1 populations with similar CD4 cell counts. As expected, X4 viruses tended to be less prevalent in newly diagnosed HIV-2 patients, naive for any antiretroviral drug, as was shown in a recent French study that reported 10% rates. Hypothetically, up to two-thirds of patients with HIV-2 in our series could benefit from CCR5 antagonists as part of their antiretroviral therapy. This information is relevant given that antiretroviral drug options are quite limited for patients with HIV-2.

Furthermore, we found a good correlation between HIV-2 tropism and CD4+ cell counts and viral load, testing for viral tropism in HIV-2 patients could assist the decision when to initiate antiretroviral therapy. Individuals with X4 viruses might warrant earlier initiation of treatment based on their increased risk for faster disease progression and poor CD4 recovery with antiretroviral therapy.

Table 2. Factors associated with X4 tropism in HIV-2-infected patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>P</td>
</tr>
<tr>
<td>Older age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>1.45 0.36–5.9</td>
<td>0.6</td>
</tr>
<tr>
<td>HIV-2 RNA (log copies/mL)</td>
<td>1.79 0.94–3.4</td>
<td>0.07</td>
</tr>
<tr>
<td>CD4 count (cells/mm³)</td>
<td>0.99 0.98–1</td>
<td>0.034</td>
</tr>
<tr>
<td>Under HAART</td>
<td>0.6 0.17–2.15</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 2. Factors associated with X4 tropism in HIV-2-infected patients

Differences in CD4 counts and viral load were also recognized when comparing X4 with R5 viruses in the subset of 19 individuals on antiretroviral therapy. The median time on antiretroviral therapy in this subset of patients was 42 (15–78) months. X4 viruses were found in eight of these patients. The median CD4+ cell count in these patients compared with patients with R5 viruses was 94 (16–147) versus 184 (43–368) cells/mm³ (P = 0.041), respectively, and the median plasma HIV-2 RNA was 3.84 (3.80–4.34) versus 3.1 (2.08–4.19) log copies/mL (P = 0.07), respectively.

In multivariate analysis, the association between HIV-2 tropism and CD4+ cell counts remained significant (P = 0.044) after adjusting for age, gender, viral load and being on antiretroviral therapy (Table 2).

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Compared with patients with HIV-1, CD4 recovery after the initiation of antiretroviral therapy in patients with HIV-2 is generally poorer. This fact might be further accentuated in HIV-2-infected individuals with X4 viruses. Our findings support that along with CD4+ cell counts and viral load, testing for viral tropism in HIV-2 patients could assist the decision when to initiate antiretroviral therapy. Individuals with X4 viruses might warrant earlier initiation of treatment based on their increased risk for faster disease progression and poor CD4 recovery with antiretroviral therapy.

Acknowledgements

Members of the HIV-2 Spanish Study Group

C. Rodríguez and J. del Romero (Centro Sanitario Sandoval, Madrid); C. Tuset, G. Marcaida, M. D. Ocete and T. Tuset (Hospital General Universitario, Valencia); E. Caballero and I. Molina (Hospital Vall d’Hebron, Barcelona); A. Aguilara, J. J. Rodríguez-Calviño, D. Navarro and B. Regueiro (Hospital Conxo-CHUS, Santiago); R. Benito, J. Gil and M. Borrás (Hospital Clínico Universitario Lazaro Blesa, Zaragoza); R. Ortiz de Lejarrazu (Hospital Clínico Universitario, Valladolid); J. M. Eiros (Hospital Rio Hortega, Valladolid); C. Manzardo and J. M. Miró (Hospital Clinic-IDIBAPS, Universidad de Barcelona, Barcelona); J. Garcia and I. Paz (Hospital Cristal-Piñor, Orense); E. Calderón and M. Leal (Hospital Virgen del Rocío, Sevilla; CIBER de Epidemiología y Salud Pública); A. Vallejo, J. M. Abad, F. Dronda and S. Moreno (Hospital Ramón y Cajal, Madrid); D. Escudero (Hospital Germans Trias i Pujol, Barcelona); M. Trigo, J. Díez, P. Álvarez, S. Cortizo and M. García-Campello (Complejo Hospitalario, Pontevedra); M. Rodríguez-Iglesias (Hospital Universitario de Puerto Real, Cádiz); A. Hernández-Betancor and A. M. Martin (Hospital Insular Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria); J. M. Ramos (Hospital Universitario, Alicante); F. Gutiérrez and J. C. Rodríguez (Hospital General, Elche); C. Gómez-Hernando (Complejo Hospitalario Virgen de la Salud, Toledo); A. Guelar (Hospital Nacional de Colón, Madrid); G. Cilla and E. Pérez-Trallero (Hospital Donostia, San Sebastián); J. López-Aldeguer (Hospital La Fe, Valencia); J. Sola (Hospital de Navarra, Pamplona); L. Fernández-Pereira (Hospital San Pedro de Alcántara, Cáceres); J. Niubó (Ciudad Sanitaria de Bellvitge, Barcelona); M. Hernández-López and J. L. Hernández-Sirvent (Hospital Universitario de Canarias La Laguna, Tenerife); L. Force (Hospital General, Mataró); C. Cifuentes (Hospital Son Llàtzer, Palma de Mallorca); S. Pérez and L. Morano (Hospital de Meixoeiro, Vigo); C. Raya (Hospital del Bierzo, Ponferrada); A. González-Praetorius (Hospital Universitario, Guadalajara); J. L. Pérez, M. Peñaranda and A. Mena (Hospital Son Dureta, Mallorca); Silva Hernández Crespo (Hospital de Basurto, Bilbao); J. M. Montejo (Hospital de Cruces, Bilbao); L. Roc and A. Martínez-Sapiña (Hospital...
Miguel Servet, Zaragoza; I. Viciana (Hospital Virgen de la Victoria, Málaga); T. Cabezás, A. Lozano and J. M. Fernández (Hospital de Poniente, Almería); I. García Bermejo and G. Gaspar (Hospital Universitario de Getafe, Madrid); R. García and M. Górgolas (Fundación Jiménez Díaz, Madrid); P. Miralles and T. Aldamiz (Hospital Gregorio Marañón, Madrid); F. García (Hospital Clínico Universitario, Granada); A. Suárez (Hospital Clínico San Carlos de Madrid); A. Treviño, P. Parra, C. de Mendoza and V. Soriano (Hospital Carlos III, Madrid).

Funding
This work was supported by grants from Fundación Investigación y Educación en Sida (IES), RIS (Red de Investigación en SIDA, ISCIII-RETIC RD/12/0017/0031), EC10/277, PI10/0520, SAF2010/22232 and the European CHAIN project (FP7-223131).

Transparency declarations
None to declare.

References
4 Poveda E, Briz V, Soriano V. Enfuvirtide, the first fusion inhibitor to treat HIV infection. AIDS Rev 2005; 7: 139 – 47.