Antiviral effect of raltegravir on HTLV-1 carriers

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Background: In vitro studies support that integrase inhibitors, such as raltegravir, may inhibit human T cell lymphotropic virus type 1 (HTLV-1) replication. However, this hypothesis has not been tested in vivo.

Methods: HTLV-1-infected individuals were invited to participate in a pilot, open study that examined whether 400 mg of raltegravir twice daily could exhibit any recognizable virological effect over 12 months. Proviral DNA was measured by a real-time PCR targeting the pol region. HTLV-1 integrase sequences were obtained from peripheral blood mononuclear cells (PBMCs) at baseline and during follow-up.

Results: A total of five HTLV-1-infected individuals entered the study. All were infected with HTLV-1 subtype a. Two patients had HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), the rest being asymptomatic. The HTLV-1 proviral load was high in all cases (median 758 HTLV-1 DNA copies/10⁴ PBMCs). Following the initiation of raltegravir therapy and for up to 6 months, both of the HAM/TSP patients experienced a transient decline in the HTLV-1 proviral load (2248 to 519 and 1033 to 861 copies/10⁴ PBMCs, respectively), returning to baseline levels on subsequent determinations. No significant changes in the HTLV-1 proviral load were noticed in the three asymptomatic individuals (median proviral load of 755 copies/10⁴ PBMCs over time). A total of 20 integrase sequences could be obtained from the five patients, and no genotypic substitutions were recognized comparing baseline and follow-up specimens under raltegravir.

Conclusions: Treatment with raltegravir in HTLV-1-infected individuals does not result in a significant reduction of proviral load beyond 6 months of therapy. The lack of continuous viral replication cycles in chronic HTLV-1 carriers most likely explains our findings.

Keywords: HTLV, integrase inhibitors, antiretroviral therapy, HAM/TSP

Introduction

Human T cell lymphotropic virus type 1 (HTLV-1), the first known human retrovirus, induces two human diseases with a long latency period: HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP); and adult T cell leukaemia (ATL).1,2 The mechanism by which the virus causes these illnesses is not fully understood, although viral replication and high proviral load are important, at least for the development of HTLV-1-associated myelopathy.3,4 Therefore, it seems worthy to examine whether antiretroviral agents widely used for treating HIV-1 infection might display activity against HTLV-1 replication enzymes, such as the reverse transcriptase, protease or integrase.5–7 Furthermore, accessory gene products, such as Tax and HTLV-1 bZIP factor (HBZ), may also provide targets for chemotherapy.8 Inhibitors of these viral proteins may further prevent the development of ATL, although such treatment may not be useful when the neoplasia has already developed and rapid outgrowth of leukemic cells occurs.

Recently, in vitro studies have claimed that integrase inhibitors, such as raltegravir, which already has been approved for the treatment of HIV, might prevent the integration of HTLV-1 in models using both cell-free and cell-to-cell modes of infection.9 Nevertheless, this hypothesis has not been tested in vivo.

Patients and methods

HTLV-1 individuals belonging to the Spanish HTLV national cohort10 were invited to participate in a pilot, open study that examined whether 400 mg of raltegravir twice daily could exhibit any recognizable virological effect over 12 months. This study was approved by the Ethics Committee of the hospital and all patients gave written informed consent before enrolment in the study.

HTLV-1 proviral DNA was quantified by real-time PCR using primers and probes targeting the pol gene that have been reported elsewhere.11 DNA was extracted from 1 × 10⁶ peripheral blood mononuclear cells (PBMCs) using the QIAamp DNA Blood Mini Kit (Qiagen, Barcelona, Spain). TaqMan amplification was carried out in a final reaction volume...
of 25 µL using TaqMan Universal Master Mix II (Applied Biosystems, Foster City, CA, USA). Thermal cycling conditions consisted of an initial step of 2 min at 50°C and an activation step at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. For each run, a standard curve was generated using 10−10^6 copies of a recombinant HTLV-1 plasmid DNA that contains one HTLV-1 pol fragment (198 bp). The HTLV copy number in each clinical sample was estimated by interpolation from the plasmidic regression curve. All reactions containing 10^4, 10^3, 10^2 and 10 copies were used to generate the standard curve. All samples were run in duplicate. The results were expressed as HTLV-1 DNA copies/10^4 PBMCs.

The analysis of the HTLV integrase gene was performed using an in-house nested PCR protocol. Briefly, 10 µL of DNA was amplified using as outer primers Int-1: 5′-AGG CCC CCT TTC AGG CCC TC-3′ and Int-2: 5′-CCG ACG GGT CCT GGG CAT GC-3′ and as inner primers Int-3 5′-AGG CCC CCT TTC AGG CCC TC-3′ and Int-4: 5′-CCG GTC TTT GGC ATG CAG CT-3′. PCR involved 35 repeated cycles (94°C for 45 s, 57°C for 45 s and 72°C for 1 min) followed by incubation at 72°C for 7 min. After checking for the presence of amplicons with electrophoresis on agarose gels, purification using Montage® PCR Centrifugal Filter Devices (Montage, Madrid, Spain) was carried out. The integrase gene was then sequenced using a cycle sequencing reaction with the Rhodamine Terminator Kit (Applied Biosystems) and the automated sequencer ABI Prism 3100. Integrate sequences were analysed using SeqScape v2.5 (Applied Biosystems).

Table 1. Main characteristics of the study population

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Country of origin</th>
<th>Transmission route</th>
<th>HTLV-1 subtype</th>
<th>HTLV-1-associated symptoms</th>
<th>Baseline HTLV-1 proviral load (copies/10^4 PBMCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>female</td>
<td>Peru</td>
<td>transfusion</td>
<td>1α</td>
<td>HAM/TSP</td>
<td>2248</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>female</td>
<td>Dominican Republic</td>
<td>heterosexual</td>
<td>1α</td>
<td>HAM/TSP</td>
<td>1033</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>male</td>
<td>Ecuador</td>
<td>homosexual</td>
<td>1α</td>
<td>no</td>
<td>215</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>female</td>
<td>Peru</td>
<td>heterosexual</td>
<td>1α</td>
<td>no</td>
<td>1163</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>male</td>
<td>Colombia</td>
<td>heterosexual</td>
<td>1α</td>
<td>no</td>
<td>806</td>
</tr>
</tbody>
</table>

Results

A total of five HTLV-1 individuals entered the study. The main baseline characteristics are recorded in Table 1. All were immigrants from South America or the Caribbean and had been living in Spain for ≥1 year. Their country of origin was Peru (two), Ecuador (one), the Dominican Republic (one) and Colombia (one). Their median age was 52 years and three were women. All were infected with HTLV-1 subtype a. The mechanism of contagion was heterosexual contact (three), transfusion (one) and homosexual relationships (one). Patient 3 was co-infected with HIV-1. Two patients had HAM/TSP, whereas the rest were asymptomatic.

At baseline, the HTLV-1 proviral load was high in all cases (median 758 HTLV-1 DNA copies/10^4 PBMCs). Following the initiation of raltegravir therapy and for up to 6 months, both HAM/TSP patients experienced a transient decline in their HTLV-1 proviral load (Figure 1). However, in both cases, a return to baseline levels was seen in subsequent determinations. No improvements in clinical manifestations were recognized in this period. In the three asymptomatic individuals, no significant decrease in HTLV-1 proviral load was noticed under raltegravir therapy, being slightly increased in Patients 4 and 5.

A total of 20 HTLV-1 integrase sequences were obtained from the five patients. No genotypic substitutions were recognized comparing baseline and follow-up specimens under raltegravir therapy. Despite the low similarity found between the HIV-1 (HXB2) and HTLV-1 sequences (26.4%), the HIV-1 catalytic motif D64-D116-E152 could be mapped at HTLV-1 integrase sequences at positions 68, 125 and 161. In a similar way, positions Y143, Q148 and N155, associated with raltegravir resistance in HIV-1, could be mapped at positions Y148, S157 and N164, respectively, at the HTLV-1 integrase. No major electric or structural changes in amino acids at these sites were manifest that might compromise the inhibitory activity of raltegravir against the HTLV-1 integrase.

Discussion

Treatment with the integrase inhibitor raltegravir failed in our study to provide any recognizable benefit in five individuals infected with HTLV-1, two of whom had HAM/TSP. There was no sustained virological effect beyond 6 months of therapy. This lack of significant benefit of raltegravir in vivo on HTLV-1 carriers argues against some promising data generated in vitro. Further evidence against any antiretroviral effect of raltegravir in these HTLV-1 carriers came from the analysis of viral integrase sequences both at baseline and during follow-up,
HTLV-1 induces the proliferation of infected T lymphocytes. The host immune system eliminates most cells expressing the virus; however, during this process, clonal populations lacking the expression of one or more viral proteins are selected and escape, and begin to expand progressively. If Tax expression is maintained, another round of immune selection occurs, and this may continue for more rounds. During this time, viral genetic changes occur because of Tax. If, by the time that viral expression is no longer detected, viral genetic changes are sufficient to make replication independent of Tax expression, ATL results. HTLV-1 proviral DNA is mono- or oligoclonally integrated in ATL cells, suggesting that in particular circumstances, such as during acute HTLV-1 infection, in the setting of severe immunosuppression (i.e. advance HIV disease, transplantation, etc.) or in accidental exposure to HTLV-1-contaminated fluids, the use of antiretroviral drugs, including corticosteroids, pentoxifylline, cyclosporine and interferon, have been tested for their ability to enhance the immune control of HTLV-1-infected cells, generally with poor results.

The lack of significant continuous replication cycles of HTLV-1 in chronic carriers most likely explains why antiretroviral agents that exhibit an inhibitory effect on HTLV-1 replication in vitro do not provide any benefit in vivo. Since viral load in HTLV-1-infected individuals is mainly maintained through cell division instead of through new rounds of viral replication and the infection of new cells, free plasma viraemia is negligible, at most. Accordingly, the activity of blockers of viral replication enzymes should not be expected. Our results, however, do not preclude that in particular circumstances, such as during acute HTLV-1 infection, in the setting of severe immunosuppression (i.e. advance HIV disease, transplantation, etc.) or in accidental exposure to HTLV-1-contaminated fluids, the use of antiretroviral drugs, including raltegravir, might prove to be helpful, given that viral replication may be relevant in these situations. Unfortunately, there is scarce experience using antiretroviral drugs in these scenarios and, therefore, no recommendations can be given at this time.

More recently, the use of drugs that may promote the expression of hidden proviruses, such as histone deacetylase inhibitors (i.e. valproic acid, SAHA), have been tried in HTLV-1 carriers, generally with poor results. More promising results have been reported for purging HTLV-1-infected cells by combining histone deacetylase inhibitors and antiretroviral agents. However, these results must be validated by others, and the recent experience using this strategy in HIV infection, where it failed to show a reduction in viral reservoirs, must be taken as a note of caution.

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**Transparency declarations**

None to declare.

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**References**


