Efficacy and safety of antiretroviral regimens including raltegravir to treat HIV-infected patients with hemophilia

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Summary

When treating HIV-infected patients with hemophilia, adverse drug reactions and interactions and the effect of treatment on bleeding disorders must be considered. Raltegravir is the first HIV integrase inhibitor, but its use in patients with hemophilia is rarely reported. Nine HIV-positive patients with hemophilia were retrospectively studied with a focus on the virological response, changes in the CD4 count, the tendency to bleed, and the response to replacement therapy before and after raltegravir-based antiretroviral therapy (ART). The nine patients were highly treatment-experienced patients and they received raltegravir-based ART for at least nine months. The patients had their own reasons for changing to raltegravir-based ART. During treatment, the CD4 count increased progressively in four patients, with a median absolute increase of 233 cells/mm³, while the count stabilized in the remaining five patients. Two previous recipients of lopinavir/ritonavir (LPV/r) who failed to respond to lamivudine (3TC) + zidovudine (ZDV) + efavirenz (EFV) had a viral rebound. Genotyping indicated multidrug resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). A pattern of resistance to raltegravir was evident, including the primary mutation N155H and the secondary mutation T97A. In the two patients, the tendency to bleed decreased markedly and monthly usage of clotting factor VIII decreased significantly decreased. In the remaining seven patients, the viral load remained < 40 copies/mL, there was no evidence of an increased tendency to bleed, and no evidence of changes in the response to replacement therapy. All of the patients had a stable condition with no signs of disease progression and no serious adverse reactions. Results indicated that Raltegravir-based therapy offered a safe and well-tolerated option for HIV-positive patients with hemophilia.

Keywords: Raltegravir, hemophilia, HIV, treatment, efficacy, safety

1. Introduction

Antiviral treatment for HIV-positive patients with hemophilia presents numerous problems in terms of safety and effectiveness. Most of these patients have been infected with the hepatitis C virus (HCV) via blood or sex, and HCV causes varying degrees of liver damage, They would receive PEG-IFN treatment plus ribavirin, HIV-positive patients with hemophilia are more prone to osteoporosis (1) and abnormal lipid metabolism (2) than patients with an HIV infection alone, and their tendency to bleed varies. When antiretroviral therapy (ART) is administered, adverse drug reactions and interactions and the effect of that therapy on bleeding disorders must be considered.

The emergence of new drugs targeting new sites during the HIV replication cycle has led to tremendous changes in therapy to manage HIV over the past few years (3). Raltegravir is the first HIV integrase inhibitor. Raltegravir was originally approved for the treatment of multidrug-resistant HIV and raltegravir has been shown to be generally safe and well-tolerated (4). However,
the use of raltegravir in patients with hemophilia is rarely reported. The current study analyzed the safety and efficacy of raltegravir-based ART in patients with hemophilia.

2. Materials and Methods

2.1. Patient population and data collection

The use of raltegravir-based ART was retrospectively analyzed in HIV-positive patients with hemophilia. All of the patients examined in this study have a documented history of HIV infection (over 10 years) and tested positive for anti-HCV antibodies and/or HCV RNA. Each patient had received ART before and was switched to raltegravir-based ART for different medical reasons for at least 9 months. All of the patients were instructed to take a 400-mg tablet of raltegravir twice daily at approximately 12-hour intervals, regardless of whether a meal was scheduled or not.

Clinical data on the patients were obtained from their original records using a data abstraction sheet, and medical charts and laboratory findings were retrospectively reviewed before and during treatment with raltegravir. The specific focus of this study was on virological response and changes in the CD4 count from the baseline. Clinical status was assessed based on clinical data and efficacy was assessed based on virological and immunological outcomes.

Safety data were collected for all patients once raltegravir was initiated. Clinicians were asked to assess the potential relationship between treatment and adverse events. If adverse events were deemed to be associated with the drug, they were recorded. Moreover, the use of coagulation factor VIII was calculated to assess the tendency to bleed and the response to replacement therapy.

2.2. Analysis of drug resistance

Negative plasma viremia was defined as plasma HIV RNA below 40 copies/mL, which was the detection limit of the HIV RNA assay. Virological response was defined as two consecutive measurements of HIV RNA < 40 copies/mL at least one week apart, and virologic failure was confirmed by a rebound in HIV RNA > 40 copies/mL. After treatment failure, the genotype of the HIV was determined.

Polymerase Chain Reaction and Sequencing

HIV RNA was extracted from plasma as previously described by Boom et al. (5), followed by a reverse transcriptase polymerase chain reaction (RT-PCR) and nested PCR of fragments of the pol gene encoding the protease (PR) enzyme, the reverse transcriptase (RT) enzyme, and the integrase (IN) enzyme, as described below. One-tube RT-PCR was performed in accordance with the manufacturer’s recommendations (One Step RT-PCR; TaKaRa). After running fragments on an agarose gel, DNA fragments of the expected size were isolated and gel was extracted using the QIA quick spin gel extraction kit (QIAGEN, Valencia, CA). The forward and reverse strands of the amplified products were sequenced.

To amplify the pol gene fragments encoding the PR, RT, and IN (HXB2 positions 2068-5221, 3154 base pairs [bp]), the primers Pol-1e (5'- TGG AAA TGT GGA(G) AAG(A) GAA(G) GGA C-3', HXB2 positions 2029-2050) and Pol-x (5'- CCT GTA TGC AG(A)(A)(C) CCC AAT ATG TT-3', HXB2 positions 5241-5265) were used for 1-tube RT-PCR, followed by nested PCR using the primers Pol-3 (5'-ACT GAG AGA CAG GCT AA TTT AGG GA-3'HXB2 positions 2068-2095) and Pol-4e (5'- CTC CTA GTG GGA TRT GTA CTT CYG ARC TTA-3'HXB2 positions 5221-5192).

To determine the resistance mutations associated with drugs, the pol gene sequences were entered in the Stanford DB database (http://hivdb.stanford.edu). This program provides analysis of the sequences of the PR, RT, and IN genes of HIV in comparison to existing published sequences of these genes.

2.3. Measurement of the CD4 count

EDTA-anticoagulated blood samples were subjected to flow cytometry (BD Company, USA, CYTOMICS-FC500) at the Shanghai Public Health Clinical Center (SHAPHC) to determine the CD4 count. In this study, the baseline CD4 count was defined as the last count measured up to one month before raltegravir-based ART began.

2.4. Ethical considerations

The study was approved by the Ethics Committee of the SHAPHC. Signed informed consent was obtained from all participants in accordance with this study's protocol.

3. Results

3.1. Clinical information on patients

In this study, information on nine HIV-positive patients with hemophilia was examined at the SHAPHC. All of the patients received ART and all were regularly followed-up.

Changes in treatment regimens and patient baseline characteristics are shown in Table 1. All of the patients had a stable condition, as indicated by a viral load < 40 copies/mL, a CD4 count > 200 cells/mm³, and co-infection with HCV. None of the patients had cirrhosis or decompensated liver disease. Seven patients had received PEG-IFN plus ribavirin therapy and had achieved a sustained virologic response (SVR), the remaining two patients being treated. Information on
Table 1. Baseline characteristics of the 9 patients examined in this study

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>HIV RNA* (copies/mL)</th>
<th>CD4+ cell count before*/after (cells/mm³)</th>
<th>HCV RNA* (copies/mL)</th>
<th>Anti-HCV*</th>
<th>ALT* (U/L)</th>
<th>Cr* (mmol/L)</th>
<th>TG* (mmol/L)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 40</td>
<td>442/447</td>
<td>&lt; 1,000</td>
<td>+</td>
<td>24</td>
<td>61</td>
<td>1.46</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 40</td>
<td>514/723</td>
<td>&lt; 1,000</td>
<td>+</td>
<td>65</td>
<td>86</td>
<td>1.16</td>
</tr>
<tr>
<td>3</td>
<td>&lt; 40</td>
<td>396/653</td>
<td>&lt; 1,000</td>
<td>+</td>
<td>28</td>
<td>70</td>
<td>2.87</td>
</tr>
<tr>
<td>4</td>
<td>&lt; 40</td>
<td>388/655</td>
<td>&lt; 1,000</td>
<td>+</td>
<td>14</td>
<td>56.7</td>
<td>3.19</td>
</tr>
<tr>
<td>5</td>
<td>&lt; 40</td>
<td>244/266</td>
<td>&lt; 1,000</td>
<td>+</td>
<td>52</td>
<td>63</td>
<td>1.24</td>
</tr>
<tr>
<td>6</td>
<td>&lt; 40</td>
<td>512/627</td>
<td>1.14e+6</td>
<td>+</td>
<td>41</td>
<td>78</td>
<td>0.67</td>
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<tr>
<td>7</td>
<td>&lt; 40</td>
<td>433/467</td>
<td>&lt; 1,000</td>
<td>+</td>
<td>82</td>
<td>112</td>
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</tr>
<tr>
<td>8</td>
<td>&lt; 40</td>
<td>541/555</td>
<td>2.06e+7</td>
<td>+</td>
<td>50</td>
<td>71</td>
<td>1.77</td>
</tr>
<tr>
<td>9</td>
<td>&lt; 40</td>
<td>766/729</td>
<td>&lt; 1,000</td>
<td>+</td>
<td>16</td>
<td>44</td>
<td>1.86</td>
</tr>
</tbody>
</table>

*Upon introduction of raltegravir-based antiretroviral therapy.

Table 2. Information on prior antiretroviral therapy and regimens including raltegravir

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>ART (before)</th>
<th>Reason</th>
<th>ART (including RAL)</th>
<th>Follow-up (months)</th>
<th>Degree of hemophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>EFV+3TC+ZDV</td>
<td>nightmares, severe anemia</td>
<td>RAL+3TC+TDF</td>
<td>24</td>
<td>severe</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>EFV+3TC+D4T</td>
<td>depression, insomnia, numbness</td>
<td>RAL+3TC+TDF</td>
<td>20</td>
<td>severe</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>EFV+3TC+TDF</td>
<td>severe osteoporosis</td>
<td>RAL+3TC+EFV</td>
<td>14</td>
<td>severe</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>LPV/r+3TC+TDF</td>
<td>bleeding from the psoas</td>
<td>RAL+3TC+TDF</td>
<td>12</td>
<td>severe</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>LPV/r+3TC+TDF</td>
<td>bleeding from the knee</td>
<td>RAL+3TC+TDF</td>
<td>12</td>
<td>severe</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>EFV+3TC+ZDV</td>
<td>depression</td>
<td>RAL+3TC+TDF</td>
<td>12</td>
<td>severe</td>
</tr>
<tr>
<td>7</td>
<td>41</td>
<td>EFV+3TC+ZDV</td>
<td>insomnia, anxiety</td>
<td>RAL+3TC+ZDV</td>
<td>14</td>
<td>severe</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>EFV+3TC+ZDV</td>
<td>insomnia, dizziness</td>
<td>RAL+3TC+ZDV</td>
<td>14</td>
<td>severe</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>LPV/r+3TC+TDF</td>
<td>intracranial hemorrhage</td>
<td>RAL+3TC+TDF</td>
<td>9</td>
<td>severe</td>
</tr>
</tbody>
</table>

Note: ART, antiretroviral therapy; EFV, efavirenz; 3TC, lamivudine; ZDV, zidovudine; D4T, stavudine; TDF, tenofovir; LPV/r, lopinavir/ritonavir; RAL, raltegravir.

prior ART and raltegravir-based ART is shown in Table 2. Three patients switched treatment due to severe spontaneous bleeding or an increased tendency to bleed and a lower response to replacement therapy for LPV/r, five patients had adverse reactions to EFV, such as depression, insomnia, or nightmares, and one patient had severe osteoporosis due to tenofovir (TDF). The duration of raltegravir treatment ranged from 9 to 24 months (mean: 14.6 months). The most commonly used antiretrovirals in optimum background treatment were 3TC, TDF, and EFV.

3.2. The efficacy of and adverse reactions to raltegravir-based ART

During treatment, none of the patients died, none developed AIDS-related opportunistic infections, AIDS-related tumors, or clinical symptoms of immune deficiency, and none had a lower CD4 count. In contrast, four patients had a progressive increase in their CD4 count, with an average absolute increase of 233 cells/mm³ (mean: 212 cells, range: 115-267 cells). In the other five patients, the CD4 count stabilized at the previous level (Table 1). Moreover, seven patients had a sustained viral load below 40 copies/mL after the introduction of raltegravir. However, two patients had a rebound in HIV. Both had been treated with 3TC + ZDV + EFV and both exhibited virologic failure, so the treatment was changed to 3TC + TDF + LPV/r. The subsequent load of HIV RNA was below 40 copies/mL. Due to severe bleeding and the lower response to replacement treatment the treatment regimens were changed to 3TC + TDF + raltegravir, but virologic failure occurred again, as indicated by a viral load of 2,640 copies/mL at 3 months, 11,600 copies/mL at 6 months and 22,000 copies/mL at 9 months in one patient, in the other patient, the viral load was 6,800 copies/mL 12 months after the introduction of raltegravir.

Anti-HCV therapy failed in the Patient 8 within one year of treatment, and the HCV RNA load in that patient remained at about 10^6 copies/mL. In contrast, a SVR was achieved in Patient 6.

Adverse reactions that are commonly associated with raltegravir include diarrhea, nausea, and headaches. Overall, there were no serious adverse events and no adverse reactions associated with raltegravir except for temporary joint pain and muscle soreness in two patients. There was no worsening of lipodystrophy or liver function (data not shown). After the change from LPV/r to raltegravir, three patients had less of a tendency to bleed and the response to replacement therapy improved significantly. Monthly usage of coagulation factor VIII by each patient remained at about 10^4 units/month. Due to severe bleeding and the lower response to replacement therapy the treatment regimens were changed to 3TC + TDF + raltegravir, but virologic failure occurred again, as indicated by a viral load of 2,640 copies/mL at 3 months, 11,600 copies/mL at 6 months and 22,000 copies/mL at 9 months in one patient, in the other patient, the viral load was 6,800 copies/mL 12 months after the introduction of raltegravir.

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Table 3. Antiretroviral regimens and respective integrase mutations in the two patients on a regimen including RAL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Therapeutic regimen</th>
<th>Viral load</th>
<th>Exposure time to RAL (m)</th>
<th>Primary mutation</th>
<th>Secondary mutation</th>
<th>Additional mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ZDV+3TC+EFV</td>
<td>rebound</td>
<td></td>
<td></td>
<td></td>
<td>S119R, K156N</td>
</tr>
<tr>
<td></td>
<td>3TC+TDF+LPV/r</td>
<td>&lt;40 copies/mL</td>
<td>9</td>
<td>N155H</td>
<td>T97A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3TC+TDF+RAL</td>
<td>22,000 copies/mL</td>
<td></td>
<td>S119R, K156N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ZDV+3TC+EFV</td>
<td>rebound</td>
<td></td>
<td></td>
<td></td>
<td>S119R, K156N</td>
</tr>
<tr>
<td></td>
<td>3TC+TDF+LPV/r</td>
<td>&lt;40 copies/mL</td>
<td>12</td>
<td>N155H</td>
<td>T97A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3TC+TDF+RAL</td>
<td>6,800 copies/mL</td>
<td></td>
<td>S119R, K156N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: ZDV, zidovudine; 3TC, lamivudine; EFV, efavirenz; TDF, tenofovir; LPV/r, lopinavir/ritonavir; RAL, raltegravir.

3.3. Analysis of drug resistance

Resistance mutation sites were examined in two patients after virologic failure of the first-line drug and raltegravir-based ART. The virus was resistant to NRTIs (resistance mutations: A62V, D67N, K70R, V75I, M184V, and K219E) and NNRTIs (resistance mutations: V90I, K103EK, and G190Q). Major or minor resistance mutations to protease inhibitors (PIs) and primary or secondary resistance mutations to integrase inhibitors (INIs) were not noted, but the additional mutations S119R, K156N, and T125S were noted.

After resistance to raltegravir-based ART was noted, the NRTI resistance mutations A62V, D67N, T69N, K70R, V75I, M184V, and K219E and the NNRTI resistance mutations V90I and G190Q were detected. In contrast, the mutation K103EK was no longer evident. Analysis of raltegravir resistance profiles revealed a pattern of mutation, including a primary mutation, N155H, and a secondary mutation, T97A. The primary mutation N155H and the secondary mutation T97A were accompanied by additional mutations, S119R and K156N, in two patients (Table 3).

4. Discussion

Raltegravir is a first-in-class HIV-1 integrase inhibitor that is safe and efficacious for both initial treatment and treatment of treatment-experienced patients with HIV-1 infection. The antiviral mechanism of action of raltegravir is its inhibition of the integration of viral DNA into the host cell nucleus (6).

Although the current sample size was small and cannot accurately yield results for all patients, HIV-positive patients with hemophilia had a stable or increased CD4 count, effective antiviral efficacy, and a stable clinical status during raltegravir-based ART.

PI might cause a higher risk of bleeding in patients with hemophilia. Of the nine current patients, three had a significant tendency to bleed, severe spontaneous bleeding, and a reduced response to replacement therapy with an ART regimen including LPV/r, so that therapy was discontinued. After the change to raltegravir-based ART, the patients had less tendency to bleed and a significantly improved response to coagulation factor VIII treatment. The monthly use of factor VIII decreased substantially.

Two patients had a virological rebound after raltegravir-based ART, so the virus infecting these patients presumably had resistance mutations. The virus was resistant to NRTIs and NNRTIs but sensitive to PI. No primary or secondary mutations were noted but additional mutations were noted when strains that were resistant to first-line drugs were genotyped. The additional mutations are associated with resistance to several INIs, both in vitro and vivo, but whether they are associated with clinical resistance to raltegravir is unclear (7-9).

Primary mutations in the catalytic domain of integrase reduced the susceptibility of HIV to raltegravir through three dependent pathways: Q148R/K/H, N155H, and Y143R/C. Secondary mutations can further increase the extent of raltegravir resistance and improve viral fitness in some cases. Analysis of resistance mutations to raltegravir revealed a pattern of mutation, including a primary mutation, N155H, and a secondary mutation, T97A, in two patients.

Several studies have indicated that a few drug-resistant HIV-1 strains present at the baseline, detectable by highly sensitive genotyping, might play an important role in the occurrence of virologic failure in patients treated with PIs or RT inhibitors (10,11). However, the link between baseline mutations and future raltegravir resistance has not been confirmed. A potential limitation of the current study is that population sequencing was used. Primary resistance mutations could be detected through use of a more sensitive method. Secondary and additional mutations are detected more frequently in baseline samples from therapy-naive and treatment-experienced patients (12-14). The frequency of all detected mutations was < 1% of the viral population, but the frequency of variation was similar in patients that responded to raltegravir and patients that did not respond to raltegravir, suggesting that these low-frequency resistance mutations do not significantly result in treatment failure. However, a point worth noting is that more secondary mutations were found at the baseline in the patients who failed to respond to treatment than in patients who responded to treatment, although the difference in the frequency of mutations was not statistically significant. Secondary mutations at the baseline were associated only with the appearance
of primary resistance mutations and served to identify patterns of mutations. Pre-existing minor mutations associated with resistance to raltegravir can appear in a large proportion of the viral population under drug selection pressure in a small subset of patients who do not respond to treatment.

The viral load was < 40 copies/mL and the virus was resistant to NRTIs and NNRTIs in two patients. Additional mutations in the virus were present before raltegravir-based ART began, and antiviral treatment of those two patients indicated that additional mutations may be related to raltegravir resistance or pre-existing mutations associated with resistance to raltegravir may appear in the viral population under drug selection pressure. To understand this issue, a longitudinal follow-up of a large number of patients treated with raltegravir needs to be conducted using highly sensitive methods.

Raltegravir-based ART was generally well-tolerated, and transaminase levels, kidney function, and TG levels were not affected by raltegravir administration. Only two patients suffered muscle aches and joint pain clearly related to raltegravir. In addition, the frequency of bleeding and bleeding patterns did not change and the response to replacement therapy did not decrease in patients who had not received LPV/r before.

Conclusion: Raltegravir-based therapy is safe, it appears in the viral population under drug selection pressure. To understand this issue, a longitudinal follow-up of a large number of patients treated with raltegravir needs to be conducted using highly sensitive methods.

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References


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