PRIMARY HIV-1 RESISTANCE – PERSISTENCE OF TRANSMITTED DRUG RESISTANCE MUTATIONS

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Abstract - Transmitted drug resistance (TDR) is one of the consequences of the high variability of HIV-1. The widespread use of antiretroviral therapy for the treatment of HIV-1 infection results in a large circulating pool of resistant virus variants. It is known that TDR mutations can persist for extended periods and may pose an important problem to the overall success of antiretroviral therapy. Factors that determine the duration of continuous persistence of resistance-associated mutations are the number and type of these mutations and their impact on viral fitness. Here we describe the follow-up of a case study of prolonged persistence of resistance-associated mutations, namely RT mutations Q151M, K65KR and Y181C conferring an intermediate-to-high level resistance to multiple NRTIs and NNRTIs that lasted for seven years. The infection was caused by subtype G virus.

Key words: HIV-1, primary resistance, Q151M complex, viral fitness, persistence

INTRODUCTION

Transmitted drug resistance (TDR) is one of the consequences of the high variability of human immunodeficiency virus type 1 (HIV-1) (Martinez-Picado and Martinez, 2008; Sanabani et al., 2011). The main characteristics of this RNA virus are its enormous genetic variability (3 x 10^-5 mutations per nucleotide per replication cycle) (Mansky, 1996) and rapid turnover in vivo (10.3x10^9 particles per day) (Perelson et al., 1996). The widespread use of antiretroviral therapy for the treatment of HIV-1 infection results in a large circulating pool of resistant virus variants (Vandamme et al., 2011; Wittkop et al., 2011). Another consequence of the genetic diversity of HIV-1 is the existence of several genetically distinct groups (M, N, O, P). In particular, within the M group a number of pure subtypes (A to K) and circulating recombinant forms exist, with differing geographical representation (Hemelaar et al., 2011).

The three main antiretroviral drug classes in use, nucleoside reverse-transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs) and protease inhibitors (PIs), are the main causes of TDR (Chan and Kantor, 2009). Highly active antiretroviral therapy (HAART), based on a combination of three or more antiretroviral drugs (ARV), was initiated in 1996. The main aim of this therapy was to suppress the plasma viral load to undetectable levels, i.e. <50 copy HIV RNA/ml plasma (Ceccherini-Silberstein et al., 2010). Implementation of HAART has significantly improved life quality and reduced morbidity and mortality of HIV infected patients in the last decades (Gallego et al., 2003; Lemey et al., 2005; Jakobsen et al., 2010). On the other hand, long-term use of this therapy has caused additional problems, of which the appearance
of drug resistance-associated mutations assumes central place (Nijhuis et al., 2001).

Considering the aforementioned, the number of studies related to the subject of transmitted drug resistance has constantly grown in the past decade (van de Vijver et al., 2006; Sagir et al., 2007; Smith et al., 2007; Wittkop et al., 2011). The results of these studies indicate that the overall prevalence of TDR was increasing in developed countries until a few years ago. Recent data appoints to the stabilization of the number of patients infected with HIV-1 resistant strains. According to data from the SPREAD program, covering the period from September 2002 through December 2005, the overall prevalence of TDR was 8.4% in Europe (Vercauteren et al., 2009). These results are similar to those published in previous reports of SPREAD program (during 1996-2002, 10.4% and 2002-2003, 9.1%) (Wensing et al., 2005; SPREAD program AIDS, 2008), as well as other studies (van de Vijver et al., 2006; Sagir et al., 2007; Smith et al., 2007; Wittkop et al., 2011). Existing data obtained over a period of 10 years have confirmed the stabilization in the number of patients who are infected with resistant strains. Moreover, several studies indicate that some risk behaviors, such as men who have sex with men (MSM), are related to the higher prevalence of TDR (Weinstock et al., 2004).

The transmission of drug-resistant viruses has been revealed to occur through several different routes, including heterosexual and homosexual intercourse, intravenous drug use and vertically, from mother to child (Erice et al., 1993; Veenstra et al., 1995; Boden et al., 1999). Potential sources for the transmission of primary resistance are people who do not know their infection status, patients who do not use antiretroviral therapy because of a high CD4 cell count and patients with HAART failure. The most widely used markers of primary resistance are Surveillance Drug Resistance Mutations (SDRM). In 2007, the list of SDRMs was established by the World Health Organization (WHO), and expanded further in 2009, as a result of both the emergence of new antiretroviral drugs and newly recognized resistance mutations (Bennett et al., 2009).

Within the survey of primary resistance in Serbia as part of the SPREAD program since 2002, multiple cases of resistance mutations have been revealed, among others a case of transmission of the multidrug resistance (MDR) thymidine analog mutations (TAMs) complex mutation Q151M. Here we describe the follow-up of this case, which is characterized by the persistence of primary resistance for seven years, prior to therapy initiation.

MATERIALS AND METHODS

Clinical and epidemiological data were collected with informed patient consent. Genotypic resistance testing was done on a sample taken upon diagnosis of HIV infection, and repeated subsequently, using an in-house genotypic resistance assay. HIV RNA extraction was done from 500 μl blood plasma (kept at -80˚C). After centrifugation, 220 μl of plasma was removed; the rest was used for RNA extraction with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Extracted RNA was reverse-transcribed and amplified by the nested RT-PCR protocol, using a One Step RNA PCR Kit (Qiagen, Hilden, Germany) for the outer reaction and a Taq PCR Core Kit (Qiagen, Hilden, Germany) for inner PCR reaction. The outer primer pair, HIV_AV159 and HIV_AV192, was used first. Nested reactions were performed with the primer pair HIV_AV190 and HIV_AV 191 (Table 1) (Snoeck et al., 2005). The obtained product was sequenced by dye-terminator sequencing on an ABI 310 automated DNA sequencer (Applied Biosystem, Foster City, CA, USA), using seven different primers (HIV_AV36, HIV_AV191, HIV_AV75, HIV_RVP3, HIV_AV22, HIV_AV44, HIV_AV190) (Table 1.). The obtained sequence was examined for mutations in the reverse transcriptase (RT) and protease (PR) gene using the algorithm provided by the HIV Drug Resistance Database (available at http://hivdb.stanford.edu).
RESULTS

A 21 year-old female patient (patient A) was diagnosed as HIV positive in August 2003 at the Center for HIV/AIDS, Belgrade. Her HIV status was discovered after an episode of lymphadenopathy and fever. At the time of inclusion in the study, the patient was in the CDC stage A. The plasma HIV-1 RNA levels and CD4 cell counts were 482/mm³ and 1000 c/ml, respectively. Genotypic resistance testing upon HIV infection diagnosis revealed the presence of primary resistance mutations. It contained Q151M, K65R, Y181C and other PI and RT mutations and polymorphisms (Table 2.). Detailed sequence analysis also revealed the subtype G of HIV-1.

Patient A was infected from a known source who is on ARV therapy. The source patient (patient B) was diagnosed in June 2002 and immediately began anti-TB therapy for disseminated tuberculosis. ARV therapy, including abacavir (ABC), lamivudine (3TC) and stavudine (d4T) was also initiated (Fig 1.). Five months later, in November 2002, due to virologic failure the therapy was switched to abacavir (ABC), didanosine (ddI) and lamivudine (3TC). Initial genotypic testing revealed that patient B’s pre-treatment sample was not associated with drug resistance mutations; however, an identical pattern of mutations was found in the on-treatment sample of the same patient and the recipient sample.

Subsequently, patient A was followed-up for several years; her clinical status did not progress significantly, with the CD4 cell count always >350/ml and pVL constantly in the range of log3. Periodical genotypic-resistance testing (Fig 1.) revealed the continuous presence of previously detected resistance mutations. In this case, the persistence of RT mutations Q151M, K65R and Y181C lasted for over seven years. In June 2010, the patient became pregnant, with CD4 431/ml, pVL 1702 c/ml and still detectable primary resistance mutations, seven years after diagnosis. The patient was not on an antiretroviral regime until the second trimester of pregnancy. Antiretroviral therapy was initiated in the second trimester consisting of lopinavir/ritonavir (LPN/RTN), saquinavir (SQV) and lamivudine (3TC), which led to an undetectable viral load. Finally, in December 2010, she gave birth to a healthy boy; her pVL<50, and peripheral CD4 count was 482/ml.

DISCUSSION

To the best of our knowledge, this is the first report of primary resistance caused by the Q151M complex. The infection was caused by HIV-1 subtype G. This case is characterized by its prolonged persistence of transmitted multi-drug resistance mutations, which lasted for seven years, namely RT mutations Q151M, K65R and Y181C.

Q151M, an NRTI resistance mutation, causes intermediate-to-high level resistance to zidovudine (AZT), didanosine (ddI), stavudine (d4T) and abacavir (ABC) and low-level resistance to tenofovir (TDF). With changes at the associated RT positions 75, 77, and 116, Q151M confers high-level resistance to zidovudine (AZT), didanosine (ddI), stavudine (d4T) and abacavir (ABC), intermediate resistance to tenofovir (TDF), and low-level resistance to lamivudine (3TC) and emtricitabine (FTC). K65R is also an NRTI resistance mutation that causes intermediate resistance to abacavir (ABC), didanosine (ddI), lamivudine (3TC) and emtricitabine (FTC) and low-level resistance to stavudine (d4T). The single NNRTI resistance mutation that appeared in our patient was Y181C. This drug resistant mutation causes high-level resistance to nevirapine (NVP) and intermediate-level resistance to efavirenz (EFV), etravirine (ETR) and rilpivirine (RPV).

Drug resistance-associated mutations are classified into two classes based on their features. Primary mutations are responsible for drug susceptibility reduction regardless of the reduced replicative capacity of the virus, while secondary mutations increase viral fitness with almost no effect on drug resistance (Deveraux et al., 2001). It is known that TDR mutations may persist for prolonged periods of time and may pose an important hindrance to the overall success of antiretroviral therapy. Factors that determine the duration of resistance-associated mutations are
the number and type of these mutations and their impact on viral fitness (García-Lerma et al., 2004).

The differing duration and scope of persistence of transmitted HIV-1 drug-resistance mutations in the absence of therapy has been described. Here we describe the case of a seven year prolonged persistence of resistance mutations, caused by the Q151M complex. Other studies have shown the persistence of other mutations for over two years, without antiretroviral therapy (Delaugerre et al., 2004). Phylogenetic analysis of several thousands of HIV-1 sequences containing drug-resistance mutations, revealed five different clusters in the United Kingdom (UK), originating from both drug-naïve and drug-treated patients (Hué et al., 2008). Detailed analysis of these clusters uncovered their persistence for up to eight years (Hué et al., 2008). Mathematical modeling allowed the estimation of the average duration of TDR persistence, showing that, on average, 4.1 years are required for a complete reversion of drug resistance mutation (Little et al., 2008).
A possible explanation for the persistence of primary resistance mutations may be the long-term process of reversion to wild type. Namely, the mechanisms that allow this are the genetic reversion of resistance mutation and viral recombination. Furthermore, the number of resistance-associated mutations is in correlation with the required period of time for their complete reversion (Delaugerre et al., 2004). Moreover, the patient in our study infected with a resistant HIV-1 strain had a relatively high number of CD4 cells and a low viral load. This may be the result of the low replicative capacity of the resistant virus (Brenner et al., 2002; Grant et al., 2002; Chaix et al., 2003).

The action mechanism of the Q151M complex is based on the modification of the dNTP biding site, decreasing the catalytic rate constant of incorporation of the analogue into DNA (Menéndez-Arias, 2008; Hachiya et al., 2011). These mutations lead to intermediate-to-high level resistance to multiple NRTIs. First studies have revealed that Q151M

<table>
<thead>
<tr>
<th>PRIMER</th>
<th>SEQUENCE (5'→3')</th>
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<tbody>
<tr>
<td>HIV_AV159</td>
<td>GGGGTAAATAAATAGTAAG</td>
</tr>
<tr>
<td>HIV_AV192</td>
<td>AATTGTTTACATGATTAGTG</td>
</tr>
<tr>
<td>HIV_AV190</td>
<td>GCCTACTAGGAAATGATGAC</td>
</tr>
<tr>
<td>HIV_AV191</td>
<td>CTTGATAAATTGTATGTGTTTG</td>
</tr>
<tr>
<td>HIV_AV75</td>
<td>TGACTGAGACAGGCTAATTTTTTAAGG</td>
</tr>
<tr>
<td>HIV_RVP3</td>
<td>GCAGAATACTGAGTTGATGATGG</td>
</tr>
<tr>
<td>HIV_AV22</td>
<td>CACCTGTCAACATAAT</td>
</tr>
<tr>
<td>HIV_AV44</td>
<td>TACTAGGTATGTTAATGCGT</td>
</tr>
<tr>
<td>HIV_AV36</td>
<td>CAGTACTGGATGTGGTGGTGATG</td>
</tr>
</tbody>
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Table 1. Specific primers used for detection 1500nt of pol region

Table 2. List of transmitted drug-associated mutations, as interpreted by the Stanford HIV database algorithm.

<table>
<thead>
<tr>
<th>Mutations</th>
<th></th>
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<tbody>
<tr>
<td>PI Major Resistance Mutations</td>
<td>None</td>
</tr>
<tr>
<td>PI Minor Resistance Mutations</td>
<td>None</td>
</tr>
<tr>
<td>PI Other Mutations</td>
<td>I13V, K14R, K20I, M36Qi, R41K, I64L, C67E, H69K, V82I, L89M, I93L</td>
</tr>
<tr>
<td>NRTI Resistance Mutations</td>
<td>K65R, Q151M</td>
</tr>
<tr>
<td>NNRTI Resistance Mutations</td>
<td>Y181C</td>
</tr>
</tbody>
</table>
complex mutations have the same replication fitness as the wild type of HIV-1 (Maeda et al., 1998). Further investigation has revealed that a single Q151M mutation and Q151M complex have better fitness compared to a wild-type strain (Dykes and Demeter, 2007). These results may point to the cause of the slow- and long-term reversion of Q151M complex mutations. The emergence of the Q151M complex requires the development of two intermediate mutations, Q151L and Q151K. These mutations have significant lower replication fitness compared with the wild type, creating a high genetic barrier for developing Q151M complex (Kosalaraksa et al., 1999; García-Lerma et al., 2000).

New reports indicate the existence of three evolutionary pathways of viral evolution after the transmission of drug-resistant HIV-1 to a new host. The first pathway is explained by a complete reversion of the major drug-resistant mutations to wild type, which is caused by the lower replicative capacity of strains with mutations compared to the wild type. The second mechanism proposes the appearance of atypical variants with a higher replicative capacity than transmitted drug-resistant forms. The last evolutionary pathway is explained by the persistence of transmitted drug-resistant viruses (Pingen et al., 2011). The evolutionary pathway of the HIV-1 strain described here, after transmission has led to long-term persistence of RT mutations. The proposed mechanism that allows the maintenance of primary resistance assumes that the replicative capacity of a transmitted drug-resistant strain is higher compared to a wild-type strain.

The first antiretroviral drug approved by the Food and Drug Administration (FDA), azidothymidine (AZT), belongs to the NRTI drug class (Whitcomb et al., 2003). Long-term use of these drugs represents one of the major causes of them being the most common TDRs. Broad cross-resistance among all NRTI drugs adds to the clinical relevance of these primary mutations. Q151M, as a thymidine analog mutation, has been found to be associated with multidrug resistance mutations in the reverse transcriptase gene (Shafer et al., 1994; Schmit et al., 1996). Nevertheless, this complex of mutations does not have high rate of cross-resistance and is seen rather rarely (Van Vaerenbergh et al., 2000; Masquelier et al., 2001).

The highest risk for mother-to-child transmission is in the third trimester of pregnancy and during delivery (Delaugerre et al., 2009); therefore, the third trimester of pregnancy is an indication for the initiation of ARV therapy. However, reports reveal that drug resistance was detected in 20% of infants, if they become infected (Delaugerre et al., 2009). This resistance is a consequence of either mother-to-child transmission or an infant’s exposure of ARV therapy immediately after birth.

Assessment of the primary resistance in non-B subtypes is very difficult because of limited sources. The baseline resistance of non-B infections increased from 2.0% in 1996-1998, to 3.0% in 1999-2000 and to 8.2% in 2000-2001 (Wensing et al., 2005). These figures match the increased number of patients infected with non-B subtype who are at present being undertaking therapy. The problem of primary resistance of B-subtype was apparent a few years before its appearance in non-B subtypes. This discrepancy is probably due to the different distribution of B and non-B subtypes. The predominant subtype in Western Europe and the United States is B, while in Africa, Asia and Eastern Europe widespread non-B subtypes exist. The higher prevalence of TDR in Western Europe and the United States compared to other regions is a consequence of the long history of antiretroviral therapy use in developed countries (Chan and Kantor, 2009).

Herein we report a unique case of persistence of transmitted RT Q151M complex mutations along with other important resistance mutations in a subtype G-infected patient during a seven-year period. This case confirms the necessity of resistance genotyping in both primary and chronic infections, as well as in pregnancy, in order to avoid HAART failure.

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REFERENCES


