MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

ORIGINAL ARTICLE

RESISTANCE OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 TO INTEGRASE STRAND TRANSFER INHIBITORS IN CROATIA: THE FIRST REPORT

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Abstract

Objectives: Integrase strand transfer inhibitors (INSTIs) are the latest class of antiretroviral drugs that prevent the integration of proviral DNA into the host genome. The aim of this study was to describe, for the first time, INSTI resistance mutations observed in Croatian HIV-infected patients.

Methods: The study was conducted between March 2016 and September 2018 and included 4 previously untreated patients (antiretroviral, ARV-naive) as well as 18 unsuccessfully treated HIV-infected patients (ARV-experienced) that have been tested for INSTI resistance. The genetic data on INSTI resistance was obtained by population-based sequencing of the integrase gene. Resistance analysis to other classes of antiretroviral drugs has been performed in some patients by sequencing the protease gene and a part of the reverse transcriptase HIV-1 gene.

Results: INSTI resistance mutations were not found in ARV-naive patients. Mutations associated with resistance to INSTIs have been observed in 5 of 18 (27.8%) patients failing INSTI-based ARV regiment. Resistance to INSTIs in ARV-experienced patients was attributed to major resistance mutations Q148R, N155H and E92Q that confer resistance to two INSTIs (raltegravir and elvitegravir).

Conclusions: The results of this study describe the first 5 cases of ARV-experienced HIV-1 infected patients with clinically significant resistance to INSTIs, and emphasize the need for continuous surveillance of INSTI resistance in patients experiencing virological failure to antiretroviral treatment in Croatia.

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INTRODUCTION

Croatia is a country with a centralized system of clinical care where all patients living with human immunodeficiency type 1 (HIV-1) infection are treated and monitored at the University Hospital for Infectious Diseases "Dr. Fran Mihaljevic" (UHID) in Zagreb.[1] Additionally, surveillance of HIV resistance to antiretroviral drugs (ART) has also been centralized at UHID since 2006.[2]

ART suppresses HIV-1 replication to undetectable levels and enables immunological reconstitution in treated patients.[3] Currently, there are six distinct classes of ART that can be classified based on their molecular mechanism and resistance profile: nucleoside-analogue reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), integrase inhibitors (INSTI), fusion inhibitors, and CCR5 antagonist. First-line ART consists of three or more antiretroviral drugs, usually two NRTIs in combination with one integrase inhibitor (recommended by the International AIDS Society-USA Guidelines, IAS-USA) or 3 INSTIs with boosted PI (Department of Health and Human Services Guidelines, DHHS).[4, 5]

INSTIs are the most recently approved class of ART that exhibit exceptionally favorable characteristics of antiviral drugs including a high barrier to resistance (dolutegravir, bictegravir), good tolerability and high antiviral potency. Therefore, INSTIs quickly became an essential part of first-line ART regimens

recommended by European AIDS Clinical Society (EACS) guidelines as well as by other professional societies within the field.[6, 7]

INSTIs prevent the integration of proviral DNA into the host genome by inhibiting the strand-transfer activity of HIV-1 integrase (IN).[8] Currently, there are four INSTIs approved by the Food and Drugs Administration and the European Medicines Agency: raltegravir (RAL), elvitegravir (EGV), dolutegravir (DTG) and bictegravir (BIC). As of 2015, RAL, EVG and DTG are the INSTIs available in Croatia.

Despite being highly effective in the treatment of HIV, resistance mutations occur in patients treated with the first generation of INSTIs (RAL and EGV), while data on primary resistance to these drugs (in previously untreated patients) are scarce.[9, 10] The appearance of major resistance mutations accompanied by further accumulation of secondary resistance mutations leads to the development of clinically significant resistance.[11, 12] Resistance to RAL has been extensively studied in the Benchmark trial in which the N155H, Q148H/K/R and Y143R pathways were identified.[13] Cross resistance between RAL and EVG has been described, and they are known to have a low genetic barrier which leads to the development of resistance with a single mutation.[14, 15] Reduced susceptibility to RAL is associated with at least 3 distinctive genetic pathways that are defined by a signature mutation Y143R/H/C, Q148H/K/R or N155H and one or more additional mutations (13). Major resistance mutations for EVG include T66I, E92Q, Q148H/K/R, and N155H.[14, 15] Several mutations are required in HIV integrase to confer high-level resistance to DTG. Dolutegravir has a higher genetic barrier to resistance than elvitegravir and raltegravir, but Q148H/K/R mutation in combination with other additional mutations could reduce its long-term potency.[16] The R263K mutation was the first mutation rarely found selected at time of virological failure in patients failing a first-line dolutegravir-based treatment.[17]

Current guidelines (DHHS, EACS, IAS-USA) recommend HIV-1 drug resistance testing for all HIV -1-infected patients prior to therapy initiation. Primary resistance testing to INSTIs as a part of routine clinical diagnostics is not currently recommended.[5, 7, 16] INSTI resistance testing has been introduced at the Department of Immunological and Molecular Diagnostics of UHID in 2016, and the majority of it is performed in patients with virological failure, as HIV-infected patients failing antiretroviral therapy should be carefully monitored. In clinical practice in Croatia, primary resistance to INSTIs is performed individually in ARV-naive patients. The aim of this study was to describe, for the first time, INSTI resistance mutations observed in Croatian HIV-infected patients.

MATERIAL AND METHODS

Study population

The present study was conducted between March 2016 and September 2018 and included 22 samples: 4 from ARV-naive and 18 from HIV-experienced patients failing therapy. Clinical and laboratory characteristics of the patients are presented in Table 1. The data used in this study was extracted from the Database of the Croatian Reference center for HIV/AIDS that contains information on all HIV-infected patients from Croatia. In the study period, some patients were tested more than once, and for 5 patients both primary and secondary resistance testing was available.

Table 1. Clinical and laboratory characteristics of patients enrolled in the study

Characteristics	
Patient, n (antiretroviral naive/experienced)	22 (4/18)
Gender males/females, n (%)	3/19 (13.6%/86.4%)
CD4+ count/ μL of blood, median (range)	147 (6-2,105)
HIV-1 RNA log10, median (range)	4.23 (3.42-7)
Acquisition route, n (%)	
Heterosexual contact	2 (9.1%)
Homosexual contact (male)	19 (86.4%)
Mother to child transmission	1 (4.5%)
HIV-1 subtype, n (%)	
Subtype B	21 (95.5%)
Subtype C	1 (4.5%)

n - number of individuals

Antiretroviral resistance testing

Resistance to antiretroviral drugs in HIV-infected patients was performed by population-based sequencing of genes coding for enzymes that are the molecular targets of drugs. Isolation of total viral RNA from the plasma of HIV-infected patients as well as amplification of targeted genes were performed by using ViroSeq HIV-1 Genotyping System according to the manufacturer's instructions (Applied Biosystems, USA).

The genetic data obtained by population-based sequencing of HIV- 1 integrase gene (codons 1-288) was performed by using ViroSeq™ HIV-1 Integrase Genotyping kit (Abbott Molecular, USA) on ABI Prism 310 Genetic Analyser (Applied Biosystems, USA). Resistance to NRTI, NNRTI and PI was performed by sequencing the protease gene (codons 1-99) and a part of reverse transcriptase gene (codons 1-335) by using Viroseq HIV-1 Genotyping System v2.0. (Applied Biosystems, USA).

INSTI resistance mutations were scored according to the International AIDS Society-USA Drug Resistance Mutations Group recommendations. Clinical significance of detected mutations was analyzed by using a bioinformatic tool Stanford University HIV

Drug Resistance Database (version 8.6.1.). HIV genotyping was performed by using the Rega algorithm for the use of genotypic HIV-1 resistance data (version Rega v10.0.0).

RESULTS

INSTI resistance mutations were found only in samples of ARV-experienced patients (n=5). Mutations Q148R, N155H and E92Q were detected as major resistance mutations associated with RAL and EVG. Possible resistance to DTG was detected in one sample (mutation Q148R). The overall prevalence of INSTI resistance mutations in ARV-experienced patients was 27.8% (5/18) (Table 2). Furthermore, treatment status of these five patients at resistance testing were tenofovir/emtricitabin+raltegravir (patients 1, 2, 4 and 5), and elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (patient 3).

Table 2. INSTI resistance mutation analysis in Croatian patients

Patients	Prevalence of resistance n (%)
Antiretroviral naive (n=4)	/
Antiretroviral experienced (n=18)	5 (27.8%)
INSTI mutations	
Q148R	1 (5.6%)
N155H	2 (11.1%)
E92Q	2 (11.1%)

n - number of individuals

Primary resistance testing to INSTI was available for 3 of 5 patients with INSTI resistance mutations, and no primary resistance mutations were found.

For 19/22 samples included in the study, resistance analysis to other classes of antiretroviral drugs was available.

In addition to INSTI resistance testing, samples of ARV-experienced patients with observed resistance to INSTIs were also analyzed to NRTI, NNRTI and PI resistance mutations. Four different drug resistance patterns (M184V/I, K65R, K70E, T69N) for NRTI, five different patterns for NNRTI (V90I, V106I, K10E, E138K, V179D) and one (Q58E) for PI were found (Table 3). Resistance to the NRTI class of drugs was detected in four of these samples, and in one sample (patient 5) multi-class resistance was observed.

According to the HIV-1 subtyping results, subtype B was found in 21/22 samples (95.5%). The one non-B sample was classified as subtype C.

DISCUSSION

The results of this study have shown, for the first time, the prevalence and patterns of INSTI resistance mutations observed in HIV-1 infected Croatian patients. The results of this study also emphasize that naturally occurring mutations or substitutions conferring resistance to INSTIs are rare.

The European SPREAD HIV resistance surveillance program published the results of a multicenter, cross-sectional study in which the prevalence of INSTI resistance was evaluated by population sequencing of the integrase gene to determine whether spontaneously generated INSTI-resistant mutants could be circulating as minority species.[18] No signature INSTI mutations were detected, whereas integrase substitutions were found in 14.3% individuals. These results showed that no INSTI resistant variants were circulating in Europe before the introduction of INSTIs in clinical practice in 2007.[18] On the other hand, polymorphisms that could contribute to INSTI resistance were frequent.[18]

There is limited number of studies analyzing transmitted drug resistance (in previously untreated patients) to INSTIs. Recent studies show very low prevalence of major resistance mutations (ranging between 0%-0.33%) in Austria, Veneto region of Italy, part of Canada, several African countries and USA. Accessory mutations were found in 0.66% to 8.5% of patients.[19-23]

Performing integrase genotyping before initiating INSTI therapy is not required, but resistance testing in individuals failing INSTI therapy is clinically warranted. In this study, major RAL and EVG resistance mutations (Q148R, N155H, E92Q) rates were 27.8% in ARV- experienced patients. These are the first cases of patients experiencing virological

Table 3. Drug resistance mutation patterns in ARV-experienced patients with observed resistance to INSTIs

Patient	INSTI mutations	Resistance to INSTIs	RT/PI mutations	Resistance to RT/PIs
Patient 1	Q148R	**RAL, EVG**, *DTG (PR)*	M184I, T69N, V90I, V106I	**FTC, 3TC**
Patient 2	N155H	**RAL, EVG**	M184V	**FTC, 3TC**
Patient 3	E92Q	**EVG**, *RAL (PR)*	M184V, K65R, K70E	**FTC, 3TC, ddI, TDF, d4t, ABC**
Patient 4	N155H	**RAL, EVG**	M184I, V179D	**FTC, 3TC**
Patient 5	E92Q	**EVG**, *RAL (PR)*	M184I, K101E, E138K, Q58E	**FTC, 3TC, RPV**, *ETR(PR), EFV(PR), NVP (PR*)

INSTI-integrase strand transfer inhibitors, RT-reverse transcriptase inhibitors; PI-protease inhibitors; ARV-antiretroviral; RAL-raltegravir, EVG-elvitegravir; DTG-dolutegravir; FTC-emtricitabine; 3TC-lamivudine; ddI-didanosine; TDF-tenofovir; d4T-stavudine, ABC-abacavir; RPV-rilpivirine; ETR-etravirine; EFV-efavirenz; NVP-nelvirapine; PR-possible resistance

failure with clinically significant resistance to RAL and EVG that have been detected in Croatia. Therefore, continuous surveillance of INSTI resistance in virological failures in Croatia is necessary, and resistance testing must be a routine practice in INSTI treatment management.

The results of HIV-1 resistance to NRTI, NNRTI and PI with various mutation patterns observed in the study reflect the repertoire of drugs that are available in Croatia prior to the introduction of INSTIs, and show multi-class drug resistance that regretfully includes all ARV drug class options. These results confirm that the use of INSTIs as a treatment of choice in Croatia should be encouraged.

The majority of HIV-infected patients in Croatia are infected with subtype B, but non-B subtypes have also been observed, mostly in the heterosexual patient group. The results of this study confirm a high proportion of subtype B infection among HIV-patients from Croatia.[24]

Virological failure occurs frequently in routine clinical practice and is associated with accumulation of mutations leading to increasing levels of drug resistance.[25] Drug resistance reduces therapeutic options and the results of this study emphasize the importance of selecting the appropriate therapy when managing ARV-experienced patients by taking into account resistance testing results. Due to the high genetic barrier to resistance, second generation INSTIs (e.g. DVG or BIC) are viable treatment options for ART-experienced patients failing therapy. This concept is also supported by the results of our study showing possible resistance to DVG in only one patient. In conclusion, the results of this study emphasize the need for continuous surveillance of INSTI resistance in patients experiencing virological failure to antiretroviral treatment in Croatia.

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