Original Research Article

HIV-2 treatment in Mali and Drug resistance antiretroviral profile

ABSTRACT

Aims: We evaluated different treatment regimens administered to patients infected with HIV-2 in Mali, and studied pol polymorphisms that may influence susceptibility to antiretrovirals.
Methodology We collected 57 blood samples from HIV - 2 seropositive patients in health centers in Mali (Bamako, Segou and Sikasso), including 21 treated patients and 36 untreated patients. The confirmation of the serological status and the measurement of the viral load were carried out in Brussels. Genotypic analysis of protease, reverse transcriptase and integrase was then performed on specimens with detectable viral load. The search for ARV resistance mutations and polymorphism positions was performed in comparison with reference sequences.
Results
The most used treatment regimen was ZDV-3TC-LPV/ r. Genotypic analysis of protease, reverse transcriptase and integrase was performed on 20 samples from patients with detectable viral load: 16 untreated patients and 4 treated patients. Seventy-five percent of the strains analyzed correspond to group A of HIV-2. In 2cas, mutations associated with resistance to the administered molecules were found. Three viral strains from untreated patients had NRTI or PI resistance mutations. In integrase, no mutation associated with resistance was observed.
Conclusion
The circulation of strains carrying resistance mutations to NRTIs and PIs has been observed in Mali.

Keywords: HIV-2, Antiretroviral therapy, efficacy, Mutations, Mali.

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1.INTRODUCTION

HIV infection is a major public health problem in most tropical countries, particularly in sub-Saharan Africa(1). In 2016, UNAIDS estimated nearly 36.7 million people living with HIV / AIDS worldwide, 25.8 million of them in sub-Saharan Africa(1). In Mali, according to the Demographic and Health Survey (DHS-V) conducted in 2012, the prevalence of HIV was estimated at 1.1%.(2). Prevalence of HIV-2 infection was 0.2% in the general population (2). HIV-2 is endemic in West Africa and spread to the 1980s in India and Europe(3-5). Compared with those infected with HIV-1, those infected with HIV-2 have a slower disease progression, lower transmission rates, and lower plasma viral loads(6). Most HIV-2 infections occur in developing countries, where access to antiretroviral therapy (ART) is limited. HIV-2 isolates are naturally resistant to NNRTIs and fusion inhibitors(7). There is a reduction in sensitivity for some protease inhibitors: nelfinavir, amprenavir and atazanavir (8). Few recommendations exist for the treatment of HIV-2 infection (9) and they are based on limited results. Nevertheless, some mutations appearing in the gene

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encoding reverse transcriptase (RT) have been clearly linked to treatment failure(10). New studies are useful to define which mutations appear on treatment, which others are natural variations or genetic polymorphisms and whether some of these polymorphisms have an impact on ART sensitivity. In Mali, no study on HIV-2 infection has been carried out. This study aims to determine the resistance profile of HIV-2 patients before and after antiretroviral treatment in Mali.

2. MATERIAL AND METHODS

This is a prospective study of the genotypic profile in 21 HIV-2 patients undergoing treatment and 36 untreated patients to any treatment. Our sample was exhaustive, based on the progressive inclusion of HIV-2 patients received in Mali.

2.1.Study population

Case: patients infected with HIV-2, confirmed by 2 rapid tests, under treatment for at least 6 months, over 18 years old and signed an informed consent form.

Control: Patient infected with HIV-2, confirmed by 2 rapid tests, untreated of any treatment and regularly monitored in health facilities, over 18 years old and signed an informed consent form.

Blood sampling

Blood samples were collected in Vacutainer tubes (Becton Dickinson, San Jose, California, USA). In the 4 hours following the blood test, the plasma was separated from the cells by centrifugation at 2000 g and stored at -20 ° C(11). Patient samples were taken at health centers in Bamako (National Institute for Public Health Research (INRSP), Gabriel Toure Hospital University, NGO Walé de Ségou, Kénédougou Solidarité Research Center (CERKES) in Sikasso). A second aliquot of biological material was stored at -20 ° C. These frozen samples were then sent to the AIDS Reference Laboratory of the Catholic University of Louvain (UCL) in Brussels, Belgium for an HIV-2 confirmation test, a plasma viral load and a sequencing of ARV target genes (the reverse transcriptase, protease and integrase).

2.2.Serological tests

The serological tests used in Mali for the HIV-2 screening were carried out according to the Malian algorithm (2 rapid tests in screening): the Genie® II HIV-1 / HIV-2 (Sanofi Diagnostics Pasteur, France) and the ImmunoComb® II HIV 1 & 2 BiSpot (Organics, Strasbourg, France). In Belgium, INNO-LIA HIV I / II Score (Innogenetics, Gent, Belgium) was used to determine HIV-1 or HIV-2 status. These tests were carried out in accordance with the operating procedures of the firms concerned.

Plasma viral load

The HIV-2 plasma viral load was quantified by real-time PCR, developed in the laboratory on a Lightcycler 2.0 platform (Roche Diagnostics, Penzberg, Germany). The assay as described by Ruelle et al. (12) was modified to obtain a sensitivity of 50 copies per ml of RNA.

HIV-2 genotype

Protease and Reverse Transcriptase

The viral RNA, which had previously been extracted for the measurement of plasma viral load, was used to perform an RT-PCR reaction and a PCR, based on the protocol described by Colson et al., (13)

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subsequently modified to improve the detection of group B and certain variants. The PCR products were analyzed on a 2% agarose gel containing ethidium bromide: this protocol generates a fragment of 1507bp, which covers the coding regions of protease and reverse transcriptase. In this case, two PCR reactions were carried out using 2 µl of RT-PCR product. The test as described by Ruelle et al.,(14), was used.

Integrase

An RT-PCR (nested PCR) reaction has been developed in the laboratory for the sequencing of HIV-2 integrase. This technique was described by Bercoff et al.,(15). The analysis of the sequences obtained was done using the software IDNSTM-Smartgene (Smartgene, Zug, Switzerland). The reference used for alignment is the HIV-2 ROD strain (Genbank M15390). The resistance mutations were listed according to the mutation list of ANRS version 2008 and the Rega algorithm version 8.01 (2009).

3. Results

Fifty-seven samples from HIV-2 or dual-reactive HIV-1 and HIV-2 patients were included in this analysis: 36 from untreated patients and 21 treated patients.

The demographic and clinical characteristics of these two groups of patients are detailed in Table I. Of the 21 patients treated, the combination of antiretroviral drugs was always based on PI, except one case of treatment of 3NRTI (ZDV + 3TC + ABC).). The ZDV + 3TC + LPV / r regimen was the most used (57% of cases): 17 patients received LPV / r and 3 received IDV / r. The overall safety of the treatment was good: only four cases of adverse Drug Reaction were recorded (2 cases of peripheral neuropathies, 1 case of visual disturbance and 1 case of overweight), 2 of which required a change in the treatment regimen. Nevertheless, after average treatment duration of 23.09 months, 11 patients (52%) are in virologic failure, have a detectable plasma viral load.

The viral genome from the patients was sequenced at the pol gene. Thirty-six patients had a detectable viral load, 25 of which had a plasma load greater than 1000copies / ml. A genotypic analysis could be carried out on the samples of 20 different patients. This analysis was completed for 14 in-between (PR + RT + IN), and partial for the others: either only integrase, or only protease and RT. The determination of the subtype or group of HIV-2 showed that 75% of the samples are in group A (15 patients) and 25% in group B (5 patients).

Genotypic analysis of integrase

Seventeen sequences of integrase were obtained: among these 14 are classified in group A and 3 in group B of HIV-2.

These sequences were aligned with the reference strains ROD (M15390) and EHO (U27200), respectively representing the groups A and B of HIV-2. Of the 32 positions described in HIV-1 as being associated with integrase inhibitor resistance, 25 positions were conserved and 7 positions were variable (Table II). No known primary resistance mutation, described in both HIV-1 and HIV-2, has been detected. The secondary resistance mutations of HIV-1 do not appear in our group of patients but variable positions at H51Q (2 cases); A153S (1 case). All the integrases have the same length for the samples of the group

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A, namely 293aa. This length is nevertheless variable among the samples of the group B: 287aa for one, 301aa for the two others.

Genotypic analysis of protease and reverse transcriptase

Sixteen sequences of protease and reverse transcriptase were obtained: of these 11 are classified in group A and 5 in group B of HIV-2. Mutations potentially implicated in resistance to protease inhibitors and reverse transcriptase inhibitors were found in 4 treated patients and 3 untreated patients. Among these, the primary mutation M184V, known to cause resistance to lamivudine and emtricitabine, was present in 2 treated patients, but also in two untreated patients. (Table III or IV). One of the two untreated patients carrying this mutation (code 6120) is a child born to an HIV-positive mother whose mother had been treated with ARVs: the virus transmitted from mother to child was probably carrying the resistance mutations presented in the (Table IV).

The majority of mutations potentially involved in resistance have been found in the protease. Among the patients treated, we find the association of mutations V62I, V71I, and I82F in one case, and the V71I mutation in the others (Table III). The association of mutations may explain treatment failure in patients 6011 and 6051, but does not explain the failure of treatment in patients 6050 and 6053. In naive patients, there are other mutations described in the table IV, mutations V47A and L90M, known to cause resistance to lopinavir, and saquinavir and other PI respectively. Patient 6126, carrying this L90M mutation, is a woman whose partner has been treated.

4. Discussion

We describe prospective data from 57 patients infected with HIV-2. The main demographic characteristics of our series correspond to the trends described in the literature (14, 16, 17). The proportion of men and women, mean weight, and average age were comparable between treated and untreated patients (14). Nevertheless, there are more patients whose age exceeds 40 years in the group of untreated patients. This observation can be interpreted as the highest proportion of patients who do not progress in their disease in the long term, not requiring treatment. Surprisingly, we have only one patient in stage A of the disease in our cohort: in published studies, the majority of HIV-2 patients are asymptomatic, stage A (18). In addition, 53% of untreated patients are found to be at Stage C and likely require treatment. Patients present themselves at a health care center when they are already symptomatic. It can therefore be postulated that many HIV-2 seropositive patients are not screened in Mali.

The impact of the treatment of viral replication was studied using as a marker the plasma viral load. All treated patients received a PI-containing combination, only one patient was treated with a combination of 3 NRTIs, and he was failing. After an average of 23 months of treatment, 52% of patients (11 cases) showed a detectable viral load. Only 4 patients with virologic failure could be analyzed genotypically, and had mutations associated with resistance at the level of reverse transcriptase or protease. Most patients had a detectable but often low viral load, which was insufficient for genotypic analysis based on plasma RNA. Of the 4 patient sequences treated, 2 were consistent with the loss of sensitivity to treatment administered. In order to study the genotypes of patients who could not be sequenced, an alternative is to

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use proviral DNA, amplified from dried blood drops. Although some specimens are available, this provirus analysis has not yet been performed at the time of the study. As the number of cases of adverse drug reaction recorded was relatively small (4cases, or 19% of patients), the main reason for treatment failures could be attributed to poor adherence to treatment, or possibly drug interactions in some cases. Nevertheless, we do not have systematic data on treatment adherence despite some patients reporting adverse drug Reaction.

Overall, the efficacy of the treatment is low but is comparable to that found by other authors who have used an association comprising a PI (14, 16, 17, 19). The number of patients treated was relatively limited in our study, but the associations received were homogeneous: 17 patients (81%) were treated with LPV/r associated with 3TC and ZDV or d4T. These combinations correspond to the associations that showed the best results in other studies or the efficacy of the treatment was evaluated by the plasma viral load and the number of CD4 cells (14, 16, 17). Yet, globally, there is a high percentage of treatment failure. In this work, the long-term treatment efficacy was difficult to evaluate because we did not have the data corresponding to treatment durations (12, 24 or 48 weeks) but an image at some point on various treatment starts after variable durations, averaging 23 months.

The suboptimal use of the treatments favors the appearance of resistant viruses. We did not observe many mutations of resistance among the patients in therapeutic failure. We have found mutations in reverse transcriptase that have been previously described: N69S, M184V and E219D (14, 20-22). The M184V mutation, the primary mutation responsible for resistance to 3TC, and FTC, was the most common. We have seen it in two naïve patients, but one of them was a child born to a previously treated HIV-positive mother, and the other a woman whose partner was probably treated. These data show that the transmission of a virus carrying the mutation M184V is possible and that this mutation is maintained without drug pressure. However, it has been described that this mutation induces a loss of replicative capacity for the virus(23), and thus the mutated variants do not continue without treatment with 3TC or FTC. This M184V variant transmission has already been observed in other studies (24, 25). It is not known yet which mechanism or which compensatory mutations allow a mutant M184V to persist without drug pressure. Other frequency resistance mutations such as K65R and Q151M have not been observed here. Positions involved in the functionality of reverse transcriptase have all been conserved (24, 26). Similarly, at the protease level, the regions essential for the catalytic activity of the HIV-1 protease (27) have all been conserved in the HIV-2 strains analyzed here. At the level of mutations related to resistance to protease inhibitors, the V71I mutation was the most widespread, common to the 4 cases under treatment but also found in 3 naive patients whose viruses also showed an association of mutations involved in resistance. Regarding the known primary mutations(14, 28, 29), the I82F primary mutation was observed in a patient under treatment and a naive patient, the V47A mutation in a naive patient and the L90M mutation also in a untreated patient. This suggests that PI strains are circulating in Mali.

The circulation of resistance strains, the low sensitivity towards certain protease inhibitors compared to HIV-1 (16), combined with the natural resistance to NNRTI and enfuvirtide, complicates the management

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of HIV-2 infection and double infections. The current need is to make available new molecules in the most affected countries that have been shown to be effective on HIV-2, such as darunavir, raltegravir and elvitegravir (30-35). We also sequenced the region of the integrase-encoding pol gene, with all patients in this study having never been treated with integrase inhibitors. Seventeen sequences were obtained, 14 aligning with group A and 3 with group B. No primary resistance mutations to raltegravir or elvitegravir described were detected in our patient group. Among the 32 positions known to be associated with resistance in HIV-1, 25 positions were conserved and 7 positions were variable: similar results have been described in the literature (36). These variations do not seem to influence the susceptibility of HIV-2 to integrase inhibitors (36, 37). As the therapeutic arsenal is reduced compared to HIV-1, this new class of drugs represents a new therapeutic option for patients infected with HIV-2 or co-infected HIV-1 and 2. It seems that the regions involved in the catalytic activity are well conserved (36, 38): the positions involved at the site of the binding with zinc (HHCC), the catalytic triad DDE, and the viral DNA binding domain RKK have been preserved in 100% of our samples. Other essential positions for viral DNA binding, integration and replication of HIV-1, namely the Q62, H67, N120, N144 and Q148 positions, are also conserved at the level of HIV-2 integrase, highlighting their crucial importance for enzymatic activity.

The length of the protein seems variable in HIV-2: while the integrase makes 288 amino acids in HIV-1, its length is 293 aa for the samples of group A and varies between 287 and 301 aa for the samples of the group B analyzes. Similar results were observed in a cohort of HIV-2 patients in France (36). The consequences of these variations are largely unknown but do not seem to affect the replicative capacity of the virus.

5. Conclusions

Three strains from untreated patients displayed mutations in protease and reverse transcriptase resistance, implying either that resistant viruses are circulating in Mali, or that some people are taking antiretroviral therapy without medical supervision. On the other hand, no mutation related to the resistance was observed at the level of the integrase: the integrase inhibitors could therefore represent a new therapeutic option for the HIV-2 patients in Mali.

Ethics approval and consent to participate

The study protocols were approved by the ethics committee of the Faculty of Medicine, Pharmacy and Dentistry of Bamako, the institutional Review Board (IRB) of Catholic University of Louvain under the number **09.084**.

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Abbreviations

ABC: abacavir; **AIDS:** acquired immune deficiency syndrome; **ART:** antiretroviral therapy; **DDE** : aspartic acid-aspartic acid-glutamic acid, **DNA**: DeoxyriboNucleic Acid; **D4T:** stavudine; **FTC:** emtricitabine ; **IDV** : indinavir ; **IN**: Integrase ; **HIV** : human immunodeficiency virus, **HIV-1:** human immunodeficiency virus type 1, **HIV-2:** human immunodeficiency virus type 2, **HHCC**: histidine-histidine-cysteine-cysteine ; **LPV**: Lopinavir, **NNRTIs:** non-nucleoside/nucleotide reverse transcriptase inhibitors **NRTIs:** nucleoside/nucleotide reverse transcriptase inhibitors, **PCR**: polymerase chain reaction; **PI:** protease inhibitor, **PR** :protease; **UNAIDS** : United Nations Programme on HIV/AIDS, **r**: Ritonavir; **RT**: reverse transcriptase; **RT-PCR**: reverse transcriptase polymerase chain reaction, **3TC**: lamivudine, **ZDV**: zidovudine

Table I: Demographic and clinical characteristics of 57 HIV-2 patients included in the study.

Characteristics	Patients treated (n=21)	Patients untreated (n=36)
Sex		
Male	7(33%)	14(38.8%)
Female	14(67%)	22(61.2%)
Mean Age	41.05	40.28
< 20 year	1(5%)	3(8%)
21-29 year	1(5%)	4(11%)
30-39 year	6(28%)	9(25%)
> 40 year	13(62%)	20(56%)
Mean weight	57.52kg	59.58kg
Stage CDC		
A	1(5%)	0
B	6(29%)	17(47%)
C	14(67%)	19(53%)
CD4 median		
Statut serologic		
HIV-2	18(86%)	33(92%)
HIV-1&2	3(14%)	3(8%)
Viral load		
Median	131copies/ml[0-35450]	3315copies/ml[0-351000]
undetectable<50 copies	10(48%)	11(31%)
50-1000copies	8(38%)	3(8%)
>1000 copies	3(14%)	22(61%)
Therapeutic regimen		
ABC-3TC-LPV/r	1(5%)	
ZDV-3TC-ABC	1(5%)	
D4T-3TC-IDV/r	3(14%)	
D4T-3TC-LPV/r	4(19%)	
ZDV-3TC-LPV/r	12(57%)	

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Adverses Drug Reaction		
No	17(81%)	
Yes	4(19%)	

Table II: Variability of HIV-2 integrase at positions related to resistance to integrase inhibitors in HIV-1

Resistance Mutation of HIV-1 INI	HIV-2 ROD subtype A consensus	Substitution (Number)	HIV-2 EHO, subtype B consensus	Substitution (Number)
H51Y	H	**Q(1)**	H	**Q(1)**
T66I/M	T		T	
V72I	I	**V(2)**	V	
L74I/M/A	I		I	
E92Q	E		E	
T97A	T		T	
T112I	H		H	
F121Y	F		F	
T125K	E		D	
A128T	M		M	
E138A/K	S	**T(1)**	T	
G140S/A	G		G	
Y143R/C	Y		Y	
Q146K	Q		E	
S147G	S		S	
Q148H/K/R/E	Q		Q	
V151I	V		V	
S153Y/A	A	**S(1)**	A	
M154I	M	**I(1)**	M	
N155S/H	N		N	
K156N	H		H	
E157Q	H		H	
K160D	N		N	

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G163R	S	**D(2),N(6)**	D	**S(1)**
V165I	I		I	
V201I	I		V	
I203M	M		M	
T206S	T		T	
S230R/N	G		G	
V249I	V		V	
R263K	R		R	
C280Y	G		S	**G(2)**

Table III : Treatment, genotype of 4 HIV-2 treated patients

Patient	Subtype	Treatment ART	Duration of treatment	Resistance PR	Resistance TI	Resistance IN
6011	A	D4T-3TC-IDV/r	34 month	V62V/I, V71I, **I82F**	**M184V**	
6050	A	ZDV-3TC-ABC	32 month	V71I		
6051	B	ZDV-3TC-LPV/r	58 month	V71I	**M184V**	
6053	B	D4T-3TC-LPV/r	15 month	V71I		

Table IV: Genotype of 3 naive HIV-2 patients

Patient	Subtype	Resistance PR	Resistance TI	Resistance IN
6120	A	K45R,**V47A**,V62A/T,V71I,L99F	**M184V**	
6126	B	K45R, T56A, V71I, **L90M**		
6130	A	I54M, V71I, **I82F**, L99F	**M184V**	

UNDER PEER REVIEW