HIV-1 drug-resistance mutations among newly diagnosed patients before scaling-up programmes in Burkina Faso and Cameroon

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We analysed whether mutations associated with resistance to antiretroviral (ARV) drugs circulate among treatment-naive HIV-1-infected individuals at a period when these drugs started to become more widely available in Africa. Overall, major resistance mutations in the *pol* gene, as defined by the International AIDS Society Resistance Testing-USA panel, were observed in 16 treatment-naive individuals. Eight of the 97 patients tested in Burkina Faso bore mutations conferring resistance to one drug class of ARV drugs: two to nucleoside reverse transcriptase inhibitors (NRTIs; M41L [*n*=1], M41L+T69S [*n*=1]), four to non-NRTIs (NNRTIs; V106A/V [*n*=1] and V108I [*n*=3]) and two to protease inhibitors (PIs; L33F [*n*=2]). In Cameroon, resistance mutations were identified in 8 of 102 patients: three to PIs (M46I/L [*n*=2], L33F [*n*=1]), three to NRTIs (T69N/T [*n*=1], M184V [*n*=1], A62V [*n*=1]) and two to NNRTIs (P236L [*n*=1], V108I [*n*=1]). It is important to note that not all genotypic drug-resistance algorithms give similar interpretations to the observed mutations. Population surveillance for ARV drug resistance is required and should be included in all implementation programmes.

Introduction

The implementation of highly active antiretroviral therapy (HAART) in developed countries has led to a marked drop in the mortality rate of AIDS patients. Until recently, only few people from countries with limited resources had access to antiretroviral (ARV) drugs, but the introduction of innovative and affordable ARV combinations together with many international efforts has led recently to a significant increase in access to those drugs. Treatment failure is frequently related to drug resistance, and transmission of HIV drug resistance to uninfected individuals raises serious clinical consequences, because it may compromise the response to initial therapy. Data from industrialized countries suggest that the transmission of drug-resistant HIV is an emerging public health problem. The prevalence of resistance mutations in newly infected individuals ranges between 10% and 25% in Europe and the United States and there is a trend towards an increase [1–4].

Studies in Africa in which patients received HAART with careful clinical and biological monitoring showed that drug-resistance mutations occured at comparable levels to what has been described for subtype B infected patients in Western countries [5]. However, the suboptimal monitoring of patients due to lack of adequate infrastructure and high prices of CD4 and viral load tests, raises fears that drug resistance could develop and spread quickly in countries with limited resources, rendering first-line anti-HIV drugs inefficient. This was illustrated in certain African countries, where suboptimal treatment together with inappropriate clinical and laboratory follow-up led rapidly to high levels of drug resistance [6,7].

In resource-limited countries where HIV treatment has been available, only a limited number of studies have been done on treatment-naive patients. The few published studies indicate still a low prevalence of mutations (from 0–6%) directly associated with resistance among recently diagnosed individuals [8–10].

In this study we examined the prevalence of ARV drug-resistance mutations in individuals recently diagnosed as infected with HIV-1 in two African countries: Burkina Faso and Cameroon.

Materials and methods

Study subjects

All the patients included in this study were ARV-therapy naive at the time of testing. The samples from Burkina Faso were collected in 2003 among 97 patients attending the main hospitals and treatment centres in Ouagadougou and Bobo Dioulasso. In Cameroon, 53 samples were from blood donors in 2001 and 49 from patients attending the Central Hospital in Yaounde, the capital city, in 2002. The national ethics committee from both countries approved the study protocol. Basic demographic and medical data were recorded on a standard questionnaire using study codes to maintain confidentiality. All patients included in the study were only recently identified as HIV positive.

HIV-1 genotyping and subtyping

Genotypic drug-resistance testing was done by sequence analysis of the protease and reverse transcriptase (RT) genes as previously described [6]. Briefly, viral RNA was extracted from plasma using the QIAamp Viral RNA kit (Qiagen, Courtaboeuf, France). RNA was transcribed into cDNA with the reverse primer IN3. cDNA was amplified by a nested PCR using Expand High Fidelity PCR system (Roche, France) with outer primers G25REV and IN3 and inner primers AV150 and polM4 (5′-CTATTAGCTGCCCCATCTACATA-3′). The amplified fragments, encompassing protease (99 amino acids) and RT (310 amino acids), were purified with QIAquick Gel Extraction kit (Qiagen) and directly sequenced using BigDye® Terminator V3.1 Cycle Sequencing kit (AppliedBiosystem, Courtaboeuf, France). In order to determine HIV-1 subtype/ circulating recombinant forms (CRFs), the new nucleotide sequences were aligned with the CLUSTAL W program with reference sequences, representing the different genetic subtypes/CRFs [11]. Phylogenetic trees were than constructed with the neighbour-joining method and reliability of branching orders obtained with the bootstrap approach were implemented by CLUSTAL W. The *pol* sequences were further investigated by bootscan and similarity analyses using the Simplot software to determine whether they were pure subtypes or CRFs (http://sray.med.som.jhmi.edu/).

Sequences were analysed to detect amino acid substitutions different to consensus subtype B (HXB2), especially for the presence of major mutations in protease and RT genes at positions known to be associated with drug resistance according to the last update from the the IAS Resistance Testing-USA panel (http://www.iasusa.org/resistance_mutations/index.html). Resistance mutation patterns were also analysed by three drug-resistance interpretation algorithms (ANRS 2005.7, Stanford Database version 4.1.7, Rega version 6.4.1), using a Stanford Resistance database tool, HIValg version 4.1.7 (http://hivdb6.stanford.edu/asi/deployed/hiv_central.pl?program=hivalg&action=showSequenceForm).

Results

Characteristics of the study population and HIV-1 strains

The characteristics of the studied population are shown in Table 1. In Burkina Faso, more than 70% were women, the median age was 33 years, and 30% were from patients in an advanced disease stage (World Health Organization [WHO] stage 3–4; Centres for Disease Control and Prevention [CDC] stage C). In Cameroon, no information was avalaible about sex or age for 29 of the 53 blood donors, which represent 28.4% of the total. Among the remaining patients, slightly more women than men were included and the median age was 35.5 years. Information on disease stage was only available for 36% and among them only a limited number were in an advanced clinical stage, the majority were classified in CDC stages A and B. Blood donors were apparently asymptomatic healthy adults. Blood donation in Cameroon is voluntary, unpaid and ill individuals are excluded.

Both, CRF02_AG (*n*=47; 48.5%) and CRF06_cpx (*n*=46; 47.4%) strains predominate in Burkina Faso, but subtypes A (*n*=3; 3.1%) and G (*n*=1; 1%) were also identified. CRF02_AG (*n*=60; 58.8%) also predominates in Cameroon but many other HIV-1 variants co-circulate: A (*n*=9; 8.9%), D (*n*=4; 3.9%), F2 (*n*=3; 2.9%), G (*n*=3; 2.9%), H (*n*=1; 1.0%), CRF01_AE (*n*=3; 2.9%), CRF09_cpx (*n*=2; 2.0%), CRF11_cpx (*n*=6; 5.9%), CRF13_cpx (*n*=4; 3.9%) and unique recombinants (*n*=7; 6.9%).

Detection of drug-resistance mutations

Major mutations associated with ARV resistance as identified by the IAS, were observed in 16 treatment-naive individuals (Table 2). Importantly, all individuals harboured only resistance to one pharmacological class of ARV drugs. In Burkina Faso, drug-resistance

Table 1. Clinical and biological characteristics of 199 patients recently diagnosed as infected with HIV-1

Characteristics	Burkina Faso (*n*=97)	Cameroon (*n*=102)
Sex		
Women, %	74 (76.3%)	40 (39.2%)
Men, %	23 (23.7%)	33 (32.4%)
No information	-	29 (28.4%)
Median age (IQR)	33 (19–56)	35.5 (17–61), ND (29.4%)
Median $CD4^+$ T-cell count, cells/mm^3 (IQR)	166 (4–952)	400 (4–1831), ND (52.9%)
WHO stages (%)		
1	20 (20.6%)	ND
2	46 (47.4%)	ND
3	21 (21.7%)	ND
4	10 (10.3%)	ND
CDC stages		
A	31 (31.9%)	14 (13.7%)
B	37 (38.2%)	22 (21.6%)
C	29 (29.9%)	1 (1.0%)
Undetermined	0 (0%)	65 (63.7%)

CDC, Centers for Disease Control and Prevention; IQR, interquartile range; ND, not determined; WHO, World Health Organization.

Table 2. Major mutations observed in protease and reverse transcriptase genes at positions in which changes are known to be associated with drug resistance by IAS and their interpretation by three commonly used drug-resistance interpretation algorithms

Mutation IAS (Oct 2005)	Total	Cameroon	Burkina Faso	ANRS v2005.7	HIVDB v4.1.7	Rega v6.4.1
L33F	3	1	2	(TPV)*	(TPV)	(TPV)
M46I/L	2	2	–	IDV, RTV	(all PIs)	RTV, (IDV,NFV)
M41L	1	–	1	†	(ZDV, D4T)	(ZDV)
M41L+T69S	1	–	1	†	(ZDV, D4T, DDI)	DDC, (ZDV)
T69N	1	1	–	†, ‡	†, ‡	DDI, DDC, (ZDV)
A62V	1	1	–	†	†	†
M184V	1	1	–	3TC, FTC	3TC, FTC	3TC, FTC
V106A	1	–	1	NVP, (EFV)	NVP, (EFV, DLV)	NVP, DLV, (EFV)
V108I	4	1	3	†	†	†
P236L	1	1		†, §	DLV	DLV
Total	16	8	8	3	3	6

*Drugs indicated in parentheses are associated with intermediate resistance, drugs indicated without brackets are associated with high level resistance. †Strains were reported as sensible by the algorithm. ‡No DDC interpretation. §No DLV interpretation. Nucleoside reverse transcriptase inhibitors: 3TC, lamivudine; ABV, abacavir; D4T, stavudine; DDC, zalcitabine; DDI, didanosine; FTC, emtricitabine; ZDV, zidovudine. Non-nucleoside reverse transcriptase inhibitors: DLV, delavirdine; EFV, efavirenz; NVP, nevirapine. Protease inhibitors: ATV, atazanavir; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir; TPV, tipranavir.

mutations were identified in eight patients: two patients bore mutations conferring resistance to nucleoside reverse transcriptase inhibitors (NRTIs; M41L+T69S [*n*=1] and M41L [*n*=1]), four to non-NRTIs (NNRTIs; V106A/V [*n*=1] and V108I [*n*=3]) and two to protease inhibitors (PIs; L33F [*n*=2]). Also in Cameroon, a total of eight patients with resistance mutations were identified: three to NRTIs (T69N/T [*n*=1], M184V [*n*=1] and A62V [*n*=1]), two to NNRTIs (P236L [*n*=1], V108I [*n*=1]) and three to PIs (M46I/L [*n*=2], L33F [*n*=1]).

It is important to note that not all genotypic drug-resistance algorithms give similar interpretations to the observed mutations (Table 2). For exemple, M46I/L was associated with resistance to indinavir and ritonavir for ANRS, whereas the Rega algorithm reported resistance to ritonavir only and intermediate susceptibility to three other PIs, and the Stanford algorithm concluded intermediate susceptibility to almost all PIs. Similarly, T69N/S was also not equally considered by the three algorithms. The L33F mutation, in protease,

is a major mutation for tipranavir in the International AIDS Society (IAS) mutation list, but the three algorithms reported intermediate resistance only to tipranavir. Moreover, the V108I mutation in RT was not at all taken into account by the three drug-resistance algorithms used. Obviously, these different interpretations led also to different proportions of drug-resistant strains.

Similarly as reported in many other studies on non-B HIV-1 protease sequences, we also detected many minor mutations on the protease gene: L10I/V (18.6%), K20M/R (5.0%), K20I (77.4%), K20V/L (2.0%), M36I (97.5%), M36L/T (1.5%), L63P (21.1%), L63A/H/I/L/M/N/ S/T/V (16.1%) and V77I (6.0%). Minor mutations in the RT were also observed: V118I (1.0%), V179E/T (1.5%) and G333D/E (12.6%). In addition, polymorphisms at positions of major mutations were observed in the protease gene (M46V [0.5%], V82I [5.0%] and in the RT gene (K101Q/N [1.0%] and V106I [2.0%]). Some of these mutations are associated with certain subtypes/CRFs (such as K20I, which is often present in CRF02 and V82I in subtype G) and therefore confirm previous findings on subtype/CRF specific polymorphisms [12].

Discussion

This study analysed whether mutations associated with resistance to ARVs circulate among treatment-naive HIV-1-infected individuals at a period when ARV drugs started to become widely available and provides thus useful background information for public health programmes on the situation at the onset of scaling-up programmes. The WHO recommends that in countries where ARV treatment is being expanded, HIV drug-resistance surveillance should focus on individuals recently infected with HIV [13]. In this study we tested recently diagnosed individuals, because this population group is more easily accessible and higher numbers of samples can be collected in a shorter time period. The proportion of resistant strains in this group does not directly reflect recently transmitted resistance, but is representative of all new patients likely to be evaluated for treatment by a clinician. This study demonstrates that primary resistance mutations are present in treatment-naive individuals before scale-up of ARV therapy in Africa. Overall, 8 out of 97 patients from Burkina Faso and 8 out of 102 from Cameroon harboured mutations associated with drug resistance. However, it is important to note that some mutations or combinations of mutations are not equally considered by the different algorithms, and subsequently the proportion of primary ARV resistance ranges from 1.5% to 3.0% in total. In industrialized countries and on the few reports from developing countries, genotypic resistance to NRTIs is more frequent. In developing countries NRTIs also circulate for a longer period and it is thus likely that in our study these mutations are transmitted [1,2,8,9]. Despite the very limited use of PIs in Africa, especially at the time that this study was conducted, we observed strains with the M46I/L or L33F PI mutations. In these patients, no other mutations associated with resistance to the more commonly available RT inhibitors were seen; therefore, they are most probably natural polymorphisms and not due to transmitted resistance. Moreover, we demonstrated in a recent study that presence of M46I/L in non-B strains from treatment-naive individuals is not related with decreased *in vitro* susceptibility to PIs [14]. Similarly, many minor mutations as natural polymorphisms seem to have no impact on *in vitro* or *in vivo* responses of non-B strains. However, they can significantly influence the interpretations of drug resistance by different algorithms which are developed for subtype B [5,14,15]. Six strains harboured NNRTI mutations, but there is not yet a complete agreement on the role of the V108I mutation between the IAS mutation list and the different algorithms. It will be important to elucidate the role of these mutations in more detail for the choice of future standardized drug regimes in resource-limited countries. Some studies showed that non-B strains could select other mutations associated with resistance. For example, V106M was selected by subtype C strains after NNRTI treatment [16,17]. It is thus possible that we underestimated ARV resistance due to not-yet-recognized resistance mutations.

The introduction of ARV drugs is relatively recent in Africa, but it seems that ARV-resistant viruses are present, either by transmission of resistance or as natural variants. Population surveillance for ARV drug resistance is required and should be included in all implementation programmes, especially in regions with suboptimal monitoring of ARV-treated patients [18]. The data collected by these programmes will provide important public health information for the recommendations of standardized first-line ARV regimens in resource-limited countries.

Acknowledgements

This study was supported by a grant form the National Agency for AIDS Research, France (ANRS 1257).

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Accepted for publication 22 March 2006