Antiretroviral Drug Resistance and Routine Therapy, Cameroon

Christian Laurent,* Charles Kouanfack,† Laurence Vergne,* Michèle Tardy,† Léopold Zekeng,‡§ Nathalie Noumsi,† Christelle Butel,* Anke Bourgeois,* Eitel Mpoudi-Ngolé,¶ Sinata Koulla-Shiro,† Martine Peeters,* and Eric Delaporte*

Among 128 patients routinely receiving highly active antiretroviral therapy in an HIV/AIDS outpatient clinic in Cameroon, 16.4% had drug resistance after a median of 10 months. Of these, 12.5% had resistance to nucleoside reverse transcriptase inhibitors (NRTIs), 10.2% to non-NRTIs, and 2.3% to protease inhibitors.

HIV drug resistance is a major threat to the scaling up of antiretroviral therapy (ART) in developing countries (the World Health Organization/United Nations Programme on HIV/AIDS “3 by 5” Initiative) (1), especially in Africa (2). Inadequate clinical and biological follow-up has been linked to high rates of drug resistance (>50% after 8 to 20 months) in Gabon (3), Côte d’Ivoire (4), and Uganda (5). In a recent study in public and private health care clinics in Douala, the economic capital of Cameroon, we found that the clinical and biological follow-up and drug supply were irregular and that many patients interrupted their treatment (6). Data on drug resistance in the routine care setting are urgently required to design large, effective ART programs. We describe the frequency and nature of major genotypic mutations conferring resistance to antiretroviral drugs among patients treated in a routine HIV/AIDS outpatient clinic in Yaoundé, the political capital of Cameroon.

The Study

We conducted a cross-sectional survey from January 2002 to January 2004 among HIV-1–infected patients managed at the Central Hospital. The patients had to pay for their drugs (US $23–$100 monthly) and laboratory tests (US $58–$85 per viral load assay and $19–$27 per CD4 cell count). Consequently, follow-up was often irregular. All patients who were given ART for at least 3 months were eligible for the study. Approximately 15%–20% of eligible patients refused or were not asked (physicians forgot) to participate. Blood samples were not available for 9 other patients. The Cameroon national ethics committee approved the study protocol, and patients gave their informed consent. Basic demographic and medical data were recorded on a standard questionnaire.

HIV was typed in each patient (HIV-1 group M, N, or O, or HIV-2) with an in-house enzyme-linked immunosorbent assay (ELISA) based on V3 loop peptides (7). Genotypic resistance to antiretroviral drugs was studied by sequencing the protease and reverse transcriptase genes with group M- or O-specific primers, depending on the serotyping results (8); samples that could not be typed with ELISA were tested with both group M and O primers. Briefly, viral RNA was extracted from plasma with the QIAamp Viral RNA minikit (Qiagen, Courtaboeuf, France) and reverse transcribed to cDNA by using Expand RT (Boehringer, Mannheim, Germany) and a reverse primer. An 1,800-bp fragment encompassing the protease and reverse transcriptase genes was amplified by nested polymerase chain reaction and directly sequenced with an ABI PrISM Big Dye Terminator cycle sequencing ready reaction kit (Perkin-Elmer, Roissy, France). Genetic subtypes were determined by phylogenetic tree analysis with the Clustal W program (8). The deduced amino acid sequences were compared with a reference sequence to detect mutations associated with resistance. Mutations were classified as minor or major, by using the September 2004 version of the French National Agency for Research on AIDS consensus statements on antiretroviral drug resistance (http://www.hivfrenchresistance.org). A susceptible strain based on absence of major drug resistance mutations by genotyping or a strain that could not be amplified for genotyping was considered nonresistant.

One hundred twenty-eight HIV-1–infected patients received ART for a median of 10 months (interquartile range [IQR] 7–18). Median age was 39 years (IQR 33–46); 70 (54.7%) of the patients were women. In addition to nucleoside reverse transcriptase inhibitors (NRTIs), 94 patients (73.4%) had received non-NRTIs (59 patients received only efavirenz, 30 received only nevirapine, and 5 received both) and 53 patients (41.4%) had received protease inhibitors (PIs, 50 patients received only indinavir, 2 received only nelfinavir, and 1 received both); 19 patients had received both non-NRTIs and PIs. Two patients (1.6%) initially received only 2 NRTIs (lamivudine and didanosine for 7 months in 1 case; stavudine and didanosine for 14 months in the other). Samples from 113 patients (88.3%) reacted with group M peptides, 3 samples (2.3%) reacted with group O peptides, and 2 other samples (1.6%)
reacted with both group M and O peptides. Ten samples did not react with group M, N, or O or HIV-2 peptides. Thirty-five samples could be amplified, and all were characterized in the pol gene. The circulating recombinant form (CRF) 02-AG strain predominated (22 patients, 62.9%); the other 13 patients had subtype A (1), D (2) or F2 (3), or CRF01-AE (2), CRF02-AF/F (2), CRF11-cpx (2), or CRF13-cpx (1).

Major genotypic mutations associated with antiretroviral drugs resistance were detected in 21 patients (16.4%, 95% confidence interval 10.5–24.0). The characteristics of these patients are shown in the Table. Sixteen patients (12.5%) had resistance to NRTIs (Figure) due to the mutations M184V (15 patients), M184I (1), T215Y (1), T215F (3), K65R (2), and Q151M (1); thymidine analog mutations M41L (2), D67N (2), K70R (3), K219Q (1), and K219E (1) were also detected. Thirteen patients (10.2%) had resistance to non-NRTIs due to the mutations K103N (11), K101E (1), Y181C (1), Y188L (2), G190E (1), and P225H (2). Three patients (2.3%) had resistance to PIs due to the mutations V82A (2 patients) and N88D (1). The 2 patients treated for a time with only 2 NRTIs (patients 2-59 and 2-84, Table) had several major genotypic mutations but had received ART for 52 and 48 months, respectively.

**Conclusions**

This observational study showed that 16.4% of patients receiving ART in a routine care setting in Cameroon had drug resistance after a median of 10 months. The rate of resistance was lower than that observed in earlier studies in Côte d’Ivoire (4), Gabon (3), and Uganda (5). Several factors could explain this finding. First, a history of suboptimal therapy was rare: only 2 patients had received a 2-drug regimen, and none had received single-agent therapy. Second, 90% of our patients began receiving ART after a national consensus conference held in June 2000 had standardized the drugs supply, drugs regimen, and clinical and biological follow-up. Third, the physicians were trained and experienced in ART use. Fourth, the cost of drugs and laboratory tests has fallen in recent years in Cameroon, a

| Patient no. | Age | Sex | Antiretroviral drugs received | Months from start of ART | Drug resistance | Major genotypic mutations | Subtype pol |
|------------|-----|-----|-----------------------------|--------------------------|----------------|--------------------------|------------|
| 2-29       | 46  | F   | 3TC, ZDV, EFV               | 33                       | 3TC, FTC, EFV, NVP | M184V, K103N, P225H | CRF02-AG   |
| 2-44       | 49  | F   | 3TC, ZDV, EFV               | 10                       | 3TC, FTC, EFV, NVP | M184V, (K70R), K103N, Y188L | CRF02-AG   |
| 2-47       | 42  | M   | 3TC, ZDV, IDV               | 10                       | 3TC, FTC                 | M184V              | CRF02-AG   |
| 2-59       | 36  | F   | 3TC, ZDV, EFV, IDV          | 52                       | 3TC, ZDV, d4T, FTC, EFV, NVP | M184V, T215F, (M41L), K103N | CRF02-AG   |
| 2-66       | 36  | M   | 3TC, ZDV, d4T, ddi, EFV     | 21                       | 3TC, FTC, EFV, NVP       | M184V, K103N    | CRF02-AG   |
| 2-70       | 30  | M   | d4T, ddi, EFV               | 6                        | EFV, NVP                 | K103N             | CRF02-AG/F  |
| 2-75       | 37  | F   | 3TC, d4T, IDV               | 9                        | 3TC, FTC                 | M184V             | A          |
| 2-76       | 34  | M   | 3TC, d4T, EFV               | 10                       | 3TC, ZDV, d4T, FTC, EFV, NVP | M184V, T215Y, K103N | F2         |
| 2-84       | 51  | M   | 3TC, ZDV, d4T, ddi, NFV     | 48                       | NRTIs, NFV               | K65R, M184V, Q151M, N88D | D          |
| 2-91       | 44  | M   | 3TC, d4T, EFV, IDV          | 6                        | EFV, NVP                 | G190E             | CRF02-AG/F  |
| 2-98       | 32  | F   | d4T, ddi, IDV               | 7                        | IDV, RTV                 | V82A              | D          |
| 2-22       | 42  | M   | 3TC, ZDV, EFV               | 14                       | 3TC, FTC, EFV, NVP       | M184I, (M41L), K101E, K103N | CRF02-AG |
| 2-29       | 33  | F   | 3TC, ZDV, d4T, ddi, EFV     | 31                       | 3TC, FTC, EFV, NVP       | M184V, K103N, P225H | CRF02-AG   |
| 2-25       | 30  | F   | 3TC, ZDV, EFV, IDV          | 18                       | 3TC, FTC                 | M184V             | CRF02-AG   |
| 2-31       | 42  | M   | 3TC, d4T, IDV               | 8                        | 3TC, FTC                 | M184V             | CRF02-AG   |
| 2-33       | 41  | F   | 3TC, ZDV, IDV               | 18                       | 3TC, FTC                 | M184V             | CRF02-AG   |
| 2-35       | 58  | M   | 3TC, ZDV, IDV               | 17                       | ATV, IDV, RTV            | V82A              | CRF01-AE   |
| 2-47A      | 48  | F   | 3TC, ZDV, EFV, IDV          | 45                       | 3TC, ZDV, d4T, FTC, EFV, NVP | M184V, T215F, (D67N, K70R, K219Q), K103N, Y188L | CRF02-AG |
| 2-50       | 32  | M   | 3TC, d4T, NVP               | 6                        | 3TC, FTC, TDF, (ABC, ddi), EFV, NVP | K65R, M184V, Y181C | CRF01-AE   |
| 2-57       | 53  | M   | 3TC, ZDV, EFV               | 7                        | EFV, NVP                 | K103N             | CRF02-AG   |
| 2-71       | 50  | F   | 3TC, ZDV, d4T, ddi, EFV     | 29                       | 3TC, FTC, EFV, NVP       | M184V, T215F, (D67N, K70R, K219E), K103N | CRF02-AG |

*ART, antiretroviral therapy; 3TC, lamivudine; ZDV, zidovudine; EFV, efavirenz; FTC, emtricitabine; NVP, nevirapine; IDV, indinavir; d4T, stavudine; ddi, didanosine; NFV, nelfinavir; NRTIs, nucleoside reverse transcriptase inhibitors; ATV, atazanavir; RTV, ritonavir; TDF, tenofovir; SQV, saquinavir; ABC, abacavir. Resistances in parentheses indicate possible resistances. Mutations in parentheses indicate thymidine analogue mutations.
fact that favors adherence to therapy. Our methods could also account for the difference: our median follow-up period was substantially less than that in the studies in Gabon and Uganda, so that our patients had less time for resistance to develop, and our assumption that nonamplification was equivalent to nonresistance could have led to an undercount of resistant strains. Lower rates of resistance were achieved in pilot studies in Cameroon (9) and Senegal (10,11), thanks to measures favoring adherence to therapy, such as provision of drugs and laboratory follow-up at no cost (or for a limited charge), and psychosocial support (counseling, access to discussion groups, and active search for patients who did not attend scheduled clinical visits, biological examinations, or drug dispensing sessions).

Resistance most often involved lamivudine (12.5%; and emtricitabine, due to mutation M184V/I related to lamivudine pressure [emtricitabine was not used in Cameroon]), efavirenz, and nevirapine (10.2%). These drugs are widely used in Cameroon in either individual formulations or fixed-dose combinations (lamivudine/zidovudine, lamivudine/stavudine, lamivudine/stavudine/nevirapine, and lamivudine/zidovudine/nevirapine). The fixed-dose combination of lamivudine/stavudine/nevirapine is now the most frequently prescribed drug in Cameroon and other African countries (12). In our study, 19 patients (14.8%) had resistance to ≥1 component on this fixed-dose combination, and high rates of resistance could compromise the use of this inexpensive (US $4.5 monthly) and convenient drug. Frequent resistance to nevirapine could also compromise the use of this drug for preventing mother-child transmission (most such programs in Africa, including in Cameroon, are based on nevirapine).

Our study showed a relatively low level of resistance after a median duration of 10 months’ treatment in a routine care setting, but we could not evaluate the association of resistance with adherence, support, or prescribing practices. The differences in methods among the African cross-sectional studies of resistance, including our own and the others referenced, make comparisons among countries difficult, although some differences are likely due to prescribing practices, drug availability, support for adherence, and follow-up. More extensive prospective studies that use standardized methods could provide comparable estimates of resistance seen at specific times (e.g., 6, 12, and 24 months after ART begins) in different countries and delineate ART program factors associated with a low prevalence of resistance.

Acknowledgments

We thank all the patients and staff who participated in the study.

This study was supported by a grant from the French National Agency for Research on AIDS (ANRS 1257) and the Institut de Recherche pour le Développement.

Dr Laurent is an epidemiologist at the Institut de Recherche pour le Développement, Montpellier, France. His major interests include epidemiology and clinical research on human immunodeficiency virus infection in Africa.

References

1. World Health Organization/United Nations Programme on HIV/AIDS. WHO-UNAIDS report. Treating 3 million by 2005: making it happen. [cited 2005 Jun 16]. Available from http://www.who.int/3by5/publications/documents/en/3by5StrategyMakingItHappen.pdf
2. Stevens W, Kaye S, Corrah T. Antiretroviral therapy in Africa. BMJ. 2004;328:280–2.
3. Vergne L, Malonga-Louellet G, Mistoul I, Mavoungou R, Mansaray H, Peeters M, et al. Resistance to antiretroviral treatment in Gabon: need for implementation of guidelines on antiretroviral therapy use and HIV-1 drug resistance monitoring in developing countries. J Acquir Immune Defic Syndr. 2002;29:165–8.
4. Adjé C, Cheingsong R, Roels TH, Maurice C, Djomand G, Verbiest W, et al. High prevalence of genotypic and phenotypic HIV-1 drug-resistant strains among patients receiving antiretroviral therapy in Abidjan, Côte d’Ivoire. J Acquir Immune Defic Syndr. 2001;26:501–6.
5. Richard N, Juntilla M, Abraha A, Demers K, Paxinos E, Galovich J, et al. High prevalence of antiretroviral resistance in treated Ugandans infected with non-subtype B human immunodeficiency virus type 1. AIDS Res Hum Retroviruses. 2004;20:355–64.
6. Laurent C, Meilo H, Gueiardi-Schmid JB, Mapoure Y, Noël JM, M’Bangou M, et al. Antiretroviral therapy in public and private routine health care clinics in Cameroon: lessons from the Douala antiretroviral (DARVIR) initiative. Clin Infect Dis. 2005;41:108–11.
7. Vergne L, Bourgeois A, Mpoudi-Ngole E, Mougnutou R, Mbuaghaw J, Liegeois F, et al. Biological and genetic characteristics of HIV infections in Cameroon reveals dual group M and O infections and a correlation between SI-inducing phenotype of the predominant CRF02_AG variant and disease stage. Virology. 2003;310:254–66.

8. Vergne L, Peeters M, Mpoudi-Ngole E, Bourgeois A, Liegeois F, Toure-Kane C, et al. Genetic diversity of protease and reverse transcriptase sequences in non-subtype-B human immunodeficiency virus type 1 strains: evidence of many minor drug resistance mutations in treatment-naive patients. J Clin Microbiol. 2000;38:3919–25.

9. Bourgeois A, Laurent C, Mougnutou R, Nkoué N, Lactuock B, Ciaffi L, et al. Field assessment of generic antiretroviral drugs: a prospective cohort study in Cameroon. Antivir Ther. 2005;10:335–41.

10. Vergne L, Touré Kane C, Laurent C, Diakhaté N, Ngom Gueye NF, Gueye PM, et al. Low rate of genotypic HIV-1 drug-resistant strains in the Senegalese government initiative of access to antiretroviral therapy. AIDS. 2003;17(Suppl 3):S31–8.

11. Lanièce I, Ciss M, Desclaux A, Diop K, Mbojd F, Ndiaye B, et al. Adherence to HAART and its principal determinants in a cohort of Senegalese adults. AIDS. 2003;17(Suppl 3):S103–8.

12. Laurent C, Kouanfack C, Koulla-Shiro S, Nkoué N, Bourgeois A, Calmy A, et al. Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicentre trial. Lancet. 2004;364:29–34.

Address for correspondence: Christian Laurent, Institut de Recherche pour le Développement – UMR 145, 911 Ave Agropolis, BP 64501, 34394 Montpellier CEDEX 5, France; email: Christian.Laurent@mpl.ird.fr