Antiviral Research, 19 (1992) 353–359

AVR 00594

Response of Ethiopian human immunodeficiency virus type 1 isolates to antiviral compounds

Zenebech Wondimu[a–c], Anders Sönnerborg[a,b], Seyom Ayehunie[a,b,d], Sven Britton[b] and Örjan Strannegård[a]

[a]*Department of Virology, the Central Microbiological Laboratory of Stockholm County Council,* [b]*Department of Infectious Diseases, Huddinge Hospital, Karolinska Institute, Stockholm, Sweden,* [c]*Addis Ababa University, Medical faculty, and* [d]*Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia*

(Received 6 February 1992; accepted 30 June 1992)

Summary

Human immunodeficiency virus type 1 (HIV-1) isolates of 8 Ethiopian and 8 Swedish untreated AIDS-patients were examined for their sensitivity to 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI) and leukocyte-derived interferon-alpha (IFN-α). No significant difference in drug sensitivity was found between Ethiopian and Swedish isolates, which all were sensitive to AZT, ddI and IFN-α except for one Swedish isolate. This isolate exhibited a mutation at amino acid position 215. These results suggest that it should be possible to perform clinical trials in Ethiopia using the same dose regimens as in Sweden.

3′-Azido-3′-deoxythymidine; 2′,3′-Dideoxyinosine; Interferon; Human immunodeficiency virus; Ethiopia; Africa

3′-Azido-3′-deoxythymidine (AZT) slows down the progression of human immunodeficiency virus (HIV) infection in European and North American patients (Yarchoan et al., 1986; Richman et al., 1987). Decreased susceptibility to AZT, however, often develops (Larder et al., 1989). This has caused a need for alternative therapies and dose regimens. Other dideoxynucleosides, such as

Correspondence to: Z. Wondimu, Department of Virology, the Central Microbiological Laboratory of Stockholm County Council, P.O. Box 70740, S-107 26 Stockholm, Sweden.

2′,3′-dideoxyinosine (ddI) (Yarchoan et al., 1989), and compounds with different modes of action, such as interferon-alpha (IFN-α) (Ho et al., 1985; Berglund et al., 1991), have therefore also been used.

The African HIV epidemic displays several features that are different from those in Europe and North America. It is unknown whether there is also a difference in the sensitivity to anti-HIV compounds. The aim of the present study was to investigate the in vitro susceptibility of Ethiopian HIV-1 isolates to AZT, ddI and IFN-α.

HIV was isolated from peripheral blood mononuclear cells (PBMC) (Ehrnst et al., 1988) of 8 Ethiopian, and 8 Swedish, untreated, AIDS patients. The isolates, which were identified as type 1 by Western blot and nucleotide sequencing (Ayehunie et al., 1991), had been passaged 1–3 times in PBMC and kept at −70°C for 1–4 years. At the time of sampling, no Ethiopian patient had been treated with AZT, ddI or IFN-α. All Ethiopian patients denied sexual intercourse with European or North American individuals.

Three antiviral drugs were evaluated: AZT, ddI and leukocyte-derived IFN-α. The drugs were used at the following concentrations: AZT at 10, 1, 0.1, 0.01, or 0.001 μM; ddI at 10, 5, 2.5, 1.25, 0.625, 0.313, or 0.156 μM; IFN-α at 625, 125, 25, 5, or 1 U/ml. The amount of HIV p24 antigen in the culture supernatants was determined by a capture ELISA (Abbott Lab., Chicago, IL, USA). Virus pellets were obtained from 3 ml of the supernatants by ultracentrifugation (55000 × *g*, 30 min), disrupted by 0.1% Triton X-100 in a lysis-buffer for 15 min at 4°C, mixed on ice with a RT-cocktail and thereafter incubated for 60 min at 37°C. The RT activity was measured in a Searle analytic Inc. Delta 300 6890 liquid scintillation system. A positive control, murine leukemia virus, and negative control supernatants were included in each run.

Analyses of Ethiopian and Swedish isolates were performed in parallel. The RT activity was measured after thawing the supernatants. Before the inoculation, 10×10^6 phytohaemagglutinin-stimulated PBMC of a mixture of two Swedish blood donors were incubated overnight with the drugs. The inoculum of each isolate (9000 cpm) was incubated with the cells for 30 min. The cells were washed after each of the two incubations. The PBMC were kept in 10-ml flasks, as described earlier (Ehrnst et al., 1988). The medium, with the same concentrations of the drugs, was changed twice a week. Two positive control cultures of each isolate, and one negative, without the drugs, were included in each experiment. The viral activity was measured by the antigen ELISA and, in certain cases, by RT activity after 3 weeks. For the AZT experiments, the cultures were also analyzed after 2 weeks. The concentrations of the drugs, which gave 50% inhibition (IC_{50}) of the p24 antigen or RT levels of positive control cultures, were determined. Based on our earlier experience (data not published), we consider isolates with an IC_{50} of up to 0.05 μM to be sensitive, those with an IC_{50} between 0.05 and 1.0 μM as partly sensitive, and those with an $IC_{50} > 1$ μM as resistant.

Nucleotide sequence analysis was performed directly on double-stranded

DNA by the dideoxynucleotide chain termination method (Ayehunie et al., 1991). A 703 base pair fragment from the RT coding region of one Swedish isolate was amplified by the polymerase chain reaction. The sequence data generated include regions that contain known mutations affecting AZT and ddI susceptibility. Statistical comparisons were performed using Mann–Whitney *U*-test and Spearman rank correlation coefficient.

A dose-dependent anti-HIV-1 activity of AZT was found for the 8 Ethiopian isolates (p24 antigen/RT- median: 0.0065/0.011 μM; range: 0.001–0.1 μM, for both) (Table 1, Fig. 1). The IC_{50}'s of week 2 and 3 did not differ significantly (data not shown). The IC_{50}'s of the p24 antigen and RT production were correlated ($r_s = 0.86$, $P = 0.02$). No viral activity was found in the negative control cultures.

Seven Ethiopian isolates were sensitive for ddI, and one strain seemed to have a decreased susceptibility (p24 antigen/RT- median: 0.160/0.320 μM; range: <0.160–3.1/<0.156–3.5 μM) (Table 1). Seven Ethiopian isolates were sensitive for IFN-α, and one strain had an increased IC_{50}, which could indicate a decreased sensitivity (median: 2.0 U/ml; range: 1.0–11.8 U/ml) (Table 2).

All but one of the Swedish HIV-1 isolates were sensitive to AZT, as measured by inhibition of p24 antigen production or RT activity (median: 0.012 and 0.01 μM; range: 0.001–1.3 and 0.002–0.07 μM) (Table 1, Fig. 1). A similar pattern was found for the IC_{50}'s of ddI (p24 antigen/RT- median: 0.5/

TABLE 1

Sensitivity to 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxyinosine (ddI) of HIV-1 isolates from Ethiopia and Sweden, measured as 50% inhibition (IC_{50}) of p24 antigen and reverse transcriptase (RT)

Isolate	IC_{50} (μM) of AZT		IC_{50} (μM) of ddI	
	Antigen	RT	Antigen	RT
Ethiopian				
1	0.003	0.001	<0.156	<0.156
2	0.059	0.044	3.1	3.5
3	0.009	0.015	n.t.	n.t.
4	0.004	0.005	0.35	0.33
5	0.001	0.001	0.31	0.31
6	0.10	0.080	<0.156	n.t.
7	0.048	0.10	0.16	0.35
8	0.003	0.007	<0.156	<0.156
Swedish				
1	0.001	0.007	0.50	0.30
2	0.005	0.044	2.20	0.97
3	0.021	0.015	0.56	0.61
4	0.001	0.002	<0.156	n.t
5	1.30	n.t.	4.5	8.0
6	0.020	0.080	<0.156	<0.156
7	0.150	0.100	0.2	0.2
8	0.005	n.t.	n.t.	n.t.

n.t. = not tested.

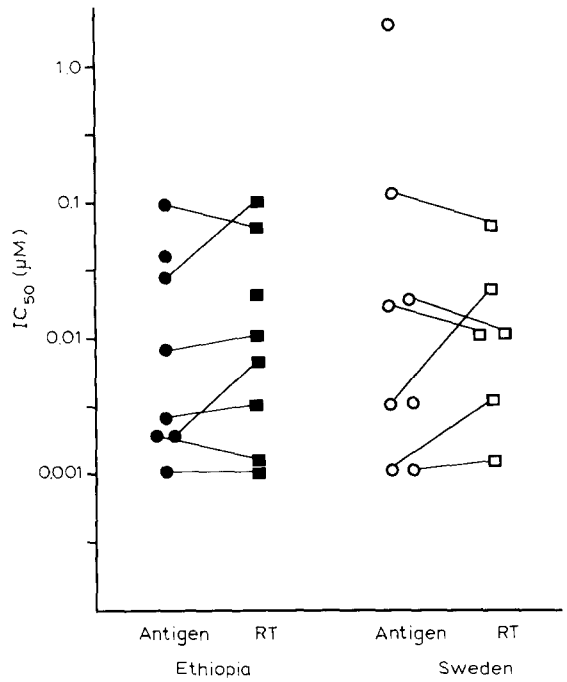

Fig. 1. Comparison between the sensitivity to AZT of Ethiopian and Swedish HIV-1 isolates, measured as 50% inhibition (IC_{50}) of the p24 antigen and reverse transcriptase (RT) production. The two IC_{50}'s of one isolate are joined by a line.

0.615 μM; range: <0.156–4.5/<0.156–8 μM). Thus, no significant difference was found between the IC_{50}'s of the Ethiopian and Swedish isolates ($P > 0.1$ for both). No major difference was found in the growth properties of the Ethiopian

TABLE 2

Sensitivity to interferon alpha (IFN-α) of HIV-1 isolates from Ethiopia and Sweden, measured as 50% inhibition (IC_{50}) of p24 antigen and reverse transcriptase (RT) production

Isolate	IC_{50} (U/ml) for IFN-α				
	Antigen	RT		Antigen	RT
Ethiopian			*Swedish*		
5	11.8	n.t.	3	56.0	87.0
2	3.4	n.t.	7	21.2	15.0
4	2.5	4.5	2	4.1	3.4
7	2.5	2.5	1	3.7	n.t.
8	2.0	n.t.	4	1.0	4.9
6	1.2	2.0	6	<1.0	n.t.
1	<1.0	n.t.			

n.t. = not tested.

and Swedish isolates, which could have affected the results of the assay (data not shown).

One Swedish isolate had an increased IC_{50} for AZT, suggesting a resistance to the compound. The isolate showed a mutation of Threonin to Tyrosin at the amino acid position 215. Although the isolate also showed reduced sensitivity to ddI, the proposed mutation at codon 74 (Leucine to Valine) could not be demonstrated. The Leucine codon used by this isolate was TTA, as compared to CTA of the HIV-SF2 isolate. One further Swedish and two Ethiopian isolates had IC_{50}'s which could possibly indicate a decreased susceptibility for AZT (0.05–1.0 μM) (Fig. 1).

Although no statistically significant difference was found between the IC_{50}'s of IFN-α for the Swedish and the Ethiopian isolates, the IC_{50}'s for the latter seemed to be lower. Thus, an IC_{50} of >3.5 U/ml was found in only 2/7 Ethiopian isolates, as compared to 5/6 Swedish isolates.

A correlation was found between the IC_{50}'s of the p24 antigen and RT production for all 3 substances when the Ethiopian and Swedish isolates were analyzed together (AZT: $r_s = 0.70$, $P = 0.015$; ddI: $r_s = 0.95$, $P = 0.003$; IFN-α: $r_s = 0.71$, $P = 0.06$).

Anti-HIV drugs are seldom used in Africa due to the lack of availability and to the high cost. However, an anti-HIV effect of AZT may also be obtained by low dose regimens (Fischl et al., 1990; Collier et al., 1990). Furthermore, no racial difference regarding the AZT effect has been found in the USA (Easterbrook et al., 1991). Therefore, it was of interest to analyze the susceptibility to AZT of Ethiopian HIV isolates. In one Swedish strain, a decreased sensitivity for AZT was associated with a mutation at amino acid position 215. It seems thus likely that the patient had been infected by an AZT-resistant strain, since he had not been treated with AZT. By contrast, no resistant Ethiopian strain was found, although some isolates showed a tendency to an increased IC_{50}. Thus, the present results suggest that Ethiopian HIV-1 strains exhibit a similar sensitivity to AZT as the Swedish strains. The same beneficial treatment effects with low-dose regimens should therefore be expected in Ethiopia as in Europe. Our results also show that ddI exhibits in vitro activity, at low concentrations, against Ethiopian isolates. Thus, ddI can be considered as an alternative to AZT in Ethiopian patients, if the cost can be reduced, e.g., by once-daily administration, which is known to improve laboratory parameters (Cooley et al., 1990).

IFN-α suppresses HIV-1 replication in vitro and in vivo (Ho et al., 1985; Berglund et al., 1991). The effect on African HIV-1 isolates in vitro has, however, not been analyzed. IFN-alpha is seldom found in the blood of Ethiopian HIV-1-infected patients (Ayehunie et al., 1992), which could theoretically lead to an increased sensitivity of Ethiopian HIV-1 strains due to a lack of selective pressure. Our results did not give any firm support for a higher sensitivity of Ethiopian HIV-1 strains. However, IFN-α inhibited the replication of the Ethiopian strains in a dose-dependent way, at concentrations which were at least as low as for the Swedish isolates (median IC_{50}: 2 versus 3.5

U/ml).

p24 antigenemia is rarely found in African patients, although virus can easily be isolated from plasma (Ayehunie et al., 1992). We therefore measured the effect on production of both p24 antigen and RT. However, no significant difference was found between the two viral markers. Thus, it seems likely that the drugs would be beneficial in vivo, even if the kinetics of viral replication is different in Ethiopian patients.

Acknowledgements

Z.W. is a recipient of a grant from the Swedish Agency for Research and Education with Developing Countries (SAREC, F/2 ETI 12). IFN-α was kindly provided by Bionative AB, Sweden.

References

Ayehunie, S., Johansson, B., Salminen, M., Leinikki, P., Sönnerborg, A., Zewdie, D., Britton, S. and Strannegård, Ö. (1991) HIV-1 in Ethiopia: phylogenetic relations to other HIV-1 strains. Virus Genes 5:4, 359–366.

Ayehunie, S., Sönnerborg, A., Desta, B., Kefene, H., Zewdie, D., Britton, S. and Strannegård, Ö. (1992) Relationship between cell-free viremia, antigenemia and antibody levels in HIV-1 infected Ethiopian patients. AIDS, 6, 651–657.

Berglund, O., Engman, K., Ehrnst, A., Andersson, J., Lidman, K., Åkerlund, B., Sönnerborg, A. and Strannegård, Ö. (1991) Combined treatment of symptomatic human immunodeficiency virus type 1 infection with native interferon-alpha and zidovudine. J. Infect. Dis. 163, 710–715.

Collier, A., Bozzette, S., Coombs, R., Causey, D., Schoenfeld, D., Spector, S., Pettinelli, C., Davies, G., Richman, D., Leedom, J., Kidd, P. and Corey, L. (1990) A pilot study of low-dose zidovudine in human immunodeficiency virus infection. N. Engl. J. Med. 323, 1015–1021.

Cooley, T., Kunches, L., Saudners, C., Ritter, J., Perkins, C., McLaren, C., Path, M., McCaffrey, R. and Liebman, H. (1990) Once-daily administration of 2′,3′-dideoxyinosine (ddI) in patients with the acquired immunodeficiency syndrome or Aids-related complex N. Engl. J. Med. 322, 1340–1345.

Easterbrook, P., Keruly, J., Creagh-Kirk, T., Richman, D., Chaisson, R. and Moore, R. (1991) Racial and ethnic differences in outcome in zidovudine-treated patients with advanced HIV disease. JAMA 266, 2713–2718.

Ehrnst, A., Sönnerborg, A., Bergdahl, S. and Strannegård, Ö. (1988) Efficient isolation of HIV from plasma during different stages of HIV infection. J. Med. Virol. 26, 23–32.

Fischl, M.A., Parker, C. and Pettinelli, C. (1990) A randomized controlled trial of a reduced daily dose of zidovudine in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. 323, 1009–1014.

Ho, D., Hartshorn, K., Rota, T., Andrews, C., Kaplan, J. and Schooley, R. (1985) Recombinant human interferon alpha-a suppresses HTLV-III replication in vitro. Lancet i, 602–604.

Larder, B.A., Darby, G. and Richman, D.D. (1989) HIV with reduced sensitivity to zidovudine isolated during prolonged therapy. Science 243, 1731–1734.

Richman, D., Fischl, M. and Grieco, M. (1987) The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N. Engl. J. Med. 317, 192–197.

Yarchoan, R., Mitsuya, H., Thomas, R., Pluda, J., Hartman, N., Perno, C., Marczyk, K., Allain, J., Johns, D. and Broder, S. (1989) In vivo activity against HIV and favourable toxicity profile of

2′,3′-dideoxyinosine. Science 245, 412–415.

Yarchoan, R., Weinhold, K., Lyerly, H., Gelmann, E., Blum, R., Shearer, G., Mitsuya, H., Collins J., Myers, C., Klecker, R., Markham, P., Durack, D., Nusinoff Lehrman, S., Barry, D., Fischl, M., Gallo, R., Bolognesi, D. and Broder, S. (1986) Administration of 3′-azido-3′-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. Lancet 1, 575–580.