

NOTES

Efficacy of Dideoxynucleosides against Human Foamy Virus and Relationship to Its Reverse Transcriptase Amino Acid Sequence and Structure

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Human foamy virus (HFV), a retrovirus of simian origin which occasionally infects humans, is the basis of retroviral vectors in development for gene therapy. Clinical considerations of how to treat patients developing an uncontrolled infection by either HFV or HFV-based vectors need to be raised. We determined the susceptibility of the HFV to dideoxynucleosides and found that only zidovudine was equally efficient against the replication of human immunodeficiency virus type 1 (HIV-1) and HFV. By contrast, zalcitabine (ddC), lamivudine (3TC), stavudine (d4T), and didanosine (ddI) were 3-, 3-, 30-, and 46-fold less efficient against HFV than against HIV-1, respectively. Some amino acid residues known to be involved in HIV-1 resistance to ddC, 3TC, d4T, and ddI were found at homologous positions of HFV reverse transcriptase (RT). These critical amino acids are located at the same positions in the three-dimensional structure of HIV-1 and HFV RT, suggesting that both enzymes share common patterns of inhibition.

Spumaviruses, in addition to oncornaviruses and lentiviruses, constitute the third genus of the *Retroviridae* family and are known to be widely prevalent in primates (15). The first spumavirus isolate found in humans was obtained in 1971 from a patient with a nasopharyngeal carcinoma (1). This isolate was named human foamy virus (HFV). The sequence data of foamy virus isolates from chimpanzees support the hypothesis that HFV is not a human prototype but rather a variant strain of a simian foamy virus (21). A substantial seroprevalence (1.8%) of infection with simian foamy virus among humans occasionally exposed to nonhuman primates was also described. These infections have not as yet resulted in either disease or sexual transmission and might represent benign endpoint infections (7). Due to its apparent lack of pathogenicity and its capacity to induce proviral genome integration in nondividing cells, HFV is used to generate retroviral vectors in development for gene therapy (8, 19, 27). Clinical considerations of how to treat patients developing an uncontrolled infection by either HFV or HFV-based vectors need to be raised. The nucleotide sequence of HFV was determined and showed that the *pol* gene was divided into three domains: reverse transcriptase (RT), RNase H, and integrase (9, 14, 17). Biological properties of the corresponding HFV *pol* gene products were determined (9). The RT of HFV might be an attractive target for efficient antiviral chemotherapy, due to the

known inhibitory effect of nucleoside analogs on human immunodeficiency virus type 1 (HIV-1) RT and their well-demonstrated activity against HIV-1 infections in vivo. In this study, we analyzed the relationship between the activity of RT dideoxynucleoside inhibitors against HFV, the RT amino acid sequence of HFV RT, and its three-dimensional (3D) structure.

Susceptibility of HFV to dideoxynucleosides. Virus stocks were obtained by infecting U373MG cells (American Type Culture Collection, Manassas, Va.) with the HFV prototype strain (kindly provided by G. Peries, Saint Louis Hospital, Paris, France) for 10 days until an 80% cytopathic effect (CPE) was observed (1). Five HIV-1 RT inhibitors were tested: azidothymidine (zidovudine [ZDV]; Sigma), dideoxyinosine (didanosine [ddI]; Sigma), dideoxycytidine (zalcitabine [ddC]; Sigma), lamivudine (3TC; Glaxo Wellcome) and stavudine (d4T; Sigma). The susceptibility of HFV to these drugs was determined by classical procedure: 2×10^4 cells per well were seeded in 96-well plates to obtain a subconfluent cell monolayer after culture for 24 h. Cells were infected in quadruplicate with 50 μ l of fivefold serial dilutions of virus stock per well. Antiviral drugs were added at the same time. The antiviral drug concentrations were those usually used for HIV-1 (4) and scaled up when 50% inhibitory concentrations (IC_{50} s) (i.e., the concentration reducing viral infectious titers by 50%) were out of range. After 10 days, plates were checked for the presence of a CPE in each well. The infectious titers in the absence and presence of drugs were calculated from the number of wells exhibiting a CPE at each concentration by the Reed and Munch method (4). All four experiments were done

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TABLE 1. Comparison of sensitivity of HFV and HIV-1 to antiviral drugs

Antiviral drug	IC ₅₀ (μM) for		IC ₅₀ ratio (HFV/HIV-1)
	HFV ^a	HIV-1	
ZDV	0.01	0.01	1
ddI	39	0.85	46
ddC	0.5	0.15	3
3TC	0.3	0.09	3
d4T	1.2	0.04	30

^a Median values of four IC₅₀ determinations are shown.

with two stocks of HFV. The susceptibility profile of HFV to an antiretroviral drug was expressed as the IC₅₀. The IC₅₀s for HFV were compared to published IC₅₀s for wild-type-sensitive HIV-1 (4) (Table 1). ZDV appeared to be the most effective drug against HFV, and its IC₅₀ was identical to that published for HIV-1. By contrast, IC₅₀s of ddC, 3TC, d4T, and ddI were 3-, 3-, 30-, and 46-fold higher, respectively, than that for HIV-1, reflecting a lower sensitivity of HFV to these drugs.

HFV sequence analysis and comparison to HIV-1 RT amino acids. We sequenced the RT active-site region of our HFV strain. The region was amplified by PCR on proviral DNA with primers RT1 (5'-CTGGTGATTATCCTCCTCGCCC-3') and RT2 (5'-AAAAGTGTCTGTTAGGCCACGACC-3') (40 cycles, each cycle at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min). A fragment of 652 bp was obtained and sequenced with an automated DNA sequencer (ABI; Perkin-Elmer). Sequence analysis with Geneworks software revealed no difference between our strain and the previously published sequence (14). To locate the homologous amino acid residues of HFV and HIV-1 RT, we performed an amino acid alignment of the deduced HFV and HIV-1 sequences with the Multalign algorithm (3), refined manually (data not shown), which confirmed those sequences previously published (14). The similarity between these proteins is low (about 20% identical amino acid residues between the two proteins). However, this limited homology extends over the entire length of both proteins, and several conserved features were revealed by the alignment. In particular, the presence of strictly conserved residues in all RNA-dependent polymerases characterized so far (18) was observed. This permitted us to investigate the relationship between the spectrum of susceptibility to dideoxynucleosides and the amino acid residues present at crucial homologous positions (Table 2). Some of the classical amino acid mutations which have been involved in the HIV-1 resistance to dideoxynucleosides are naturally present in HFV RT amino acid sequences, at the HIV-1 positions 74 and 184 for ddI and ddC (5, 6, 24), position 184 for 3TC (20, 26) and position 75 for d4T (11, 13; D. Shepp, A. Ashraf, C. Millian, and R. Pergolizzi, Abstr. 2nd Natl. Conf. Human Retrovir., abstr. 139, 1995). At other positions, HFV amino acids were different from those present in sensitive HIV-1 strains but also distinct from those observed in resistant HIV-1 strains. In the case of ZDV, which exhibited the same activity level on HFV and HIV-1, we did not find any of the amino acid residues involved in HIV-1 resistance to the drug at positions 41, 67, 70, 215, and 219 (12), corresponding to positions 91, 118, 121, 252, and 256 of the HFV RT. In addition, neither multidrug resistance mutations

(Q151 M profile) (22, 23) nor a position 69 insertion (J. J. de Jong, S. Jurriaans, J. Goudsmit, E. Baan, R. Huismans, S. A. Danner, M. E. Hillebrand, J. H. ten Veen, and F. de Wolf, Antivir. Ther., abstr. 18, 1998) of resistant HIV-1 strains was observed in the case of HFV. These results are in accordance to the susceptibility pattern of HFV to dideoxynucleosides depicted in Table 1.

HFV RT 3D structural modeling. We determined a putative 3D structural modeling of HFV RT to check if the crucial positions defined by sequence alignment were located at the same 3D positions as in HIV-1 RT. The molecular modeling of HFV was calculated by homology with the 3D structure of HIV-1 (BH10 isolate) RT (3HVT.pdb) with the Swiss Model Automated Protein Modelling service at the Glaxo Wellcome Experimental Research Center (Geneva, Switzerland), which makes use of the ProMod software (16). Secondary structure and distribution of hydrophobic clusters were analyzed as previously described (2), and the results suggested that the structural organization of both proteins was similar (data not shown). Therefore, on the basis of the alignment, we calculated the 3D structure of the HFV RT by homology with the 3D structure of HIV-1 enzyme, which has been determined by X-ray diffraction (10). This model markedly suggested that the structural topology was conserved in both proteins (Fig. 1), in particular, the organization in different subdomains (fingers, palm, thumb, and connection regions) (25). Moreover, the amino acids corresponding to the catalytic triad of aspartic acids (Asp 110, Asp 185, and Asp 186 for HIV-1 and Asp 161, Asp 223, and Asp 224 for HFV), as well as those related to dideoxynucleoside resistance (Asp 74, Asp 75, and Asp 184 for HIV-1 and Asp 124, Asp 125, and Asp 222 for HFV), are located at similar positions in the 3D structure of both proteins. This 3D representation permitted us to verify that the

TABLE 2. Amino acid residues of HIV-1 RT involved in resistance to dideoxynucleosides and correspondence to amino acid residues of HFV RT

Antiviral drug	HIV-1 residue(s)			HFV RT residue	
	Position	Sensitive strain	Resistant strain	Homologous position	Tested strain ^a
AZT	41	M	L	91	I
	67	D	N	118	D
	70	K	R	121	
	215	T	Y/F	252	V
	219	K	Q/E	256	K
ddI	65	K	R	116	K
	74	L	V	124	<u>V</u>
	75	V	T	125	<u>L</u>
	184	M	V/I	222	<u>V</u>
ddC	65	K	R	116	K
	69	T	D	120	R
	74	L	V	124	<u>V</u>
	75	V	T	125	<u>L</u>
	184	M	V/I	222	<u>V</u>
3TC	184	M	V/I	222	<u>V</u>
d4T	75	V	T/M/S/A/L	125	<u>L</u>

^a Underlined amino acids are identical in resistant HIV-1 strains and HFV.

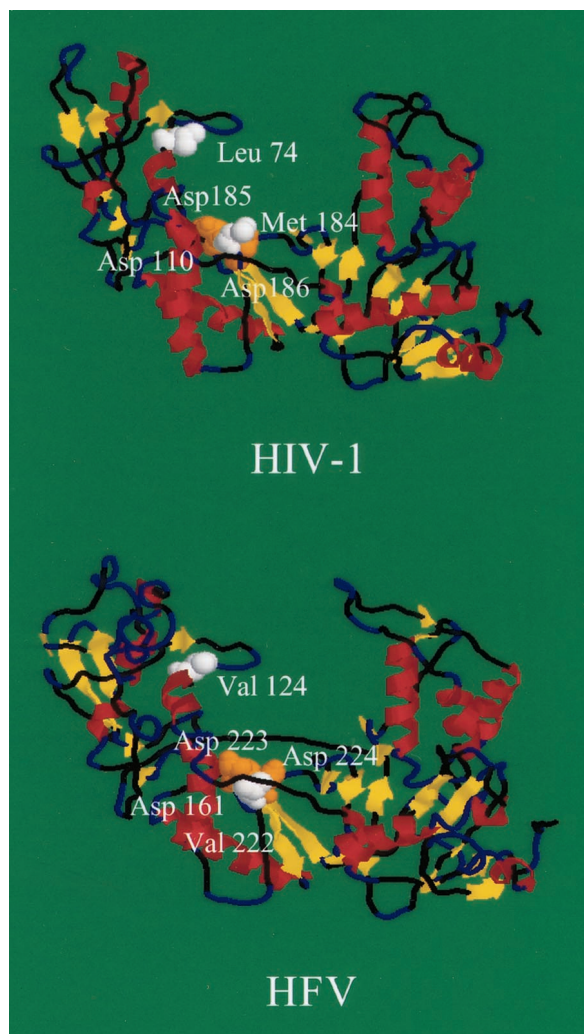


FIG. 1. Predicted 3D structure of HFV RT based on the known structure of HIV-1 RT. α -Helices are indicated in red, and β -strands are indicated in yellow. Essential aspartates (Asp 110, Asp 185, and Asp 186 for HIV-1 and Asp 161, Asp 223, and Asp 224 for HFV), which may bind the triphosphate moiety of incoming nucleotide analogs and deoxynucleoside triphosphate substrates via Mg^{2+} , are indicated as orange spheres. White spheres indicate two positions involved in resistance of HIV-1 to dideoxynucleoside (amino acids 74 and 184) and the homologous position in HFV RT.

amino acids in HFV RT corresponding to those involved in HIV-1 dideoxynucleoside resistance were located at the same 3D positions. The fact that critical amino acid residues involved in resistance are located in the same subdomains in both proteins confirms the previous results identifying the exact localization of HFV RT gene (9).

Our work studied the efficacy and the relationship between dideoxynucleoside analog susceptibility, amino acid sequence, and structure of HFV RT. We evaluated the HFV susceptibility profile of five dideoxynucleoside analogs commonly used in HIV-1 treatment. The IC_{50} values were shown to be higher than those previously described for HIV-1, except that of ZDV. These results are in accordance with those recently published (28). To correlate function inhibition and structure, we determined the RT amino acid se-

quence of the HFV strain tested, and we performed alignment with HIV-1 RT. The genomic determinants of the natural resistance pattern of HFV to the dideoxynucleosides tested were found to be homologous to those previously described for HIV-1 resistance, despite a low similarity between HFV and HIV-1 RT. A 3D model of HFV RT confirmed these results, which might have important implications in both the studies of retrovirus phylogeny and the management of HFV infections in vivo.

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