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Human Immunodeficiency Virus (HIV) Type 1 Reverse Transcriptase Resistance Mutations in Hepatitis B Virus (HBV)-HIV-Coinfected Patients Treated for HBV Chronic Infection Once Daily with 10 Milligrams of Adefovir Dipivoxil Combined with Lamivudine

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Adefovir dipivoxil (ADV) at a suboptimal concentration for human immunodeficiency virus type 1 (HIV-1) (10 mg once daily) can be used to treat hepatitis B virus (HBV) infection in HIV-1–HBV-coinfected patients and does not, even in the case of uncontrolled HIV-1 replication, select for either ADV mutations at codons 65 and 70 or any other particular HIV-1 reverse transcriptase resistance profile.

Adefovir {9-[2-(phosphonomethoxy)ethyl]adenine [PMEA]}, a phosphonate nucleoside analog of AMP, has broad-spectrum antiviral activity, targeting herpesviruses, retroviruses, and hepadnaviruses (14). Adefovir requires only two phosphorylation steps to reach its active metabolite, while the nucleoside analogs require three phosphorylation steps. The active form is a competitive inhibitor with regard to dATP and is incorporated into viral DNA, functioning as a chain terminator of viral DNA synthesis. Adefovir dipivoxil (ADV), the orally bioavailable prodrug of PMEA, has been tested in clinical trials for the potential treatment of human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV) infections. Clinical trials against HIV have shown modest antiretroviral activity and adefovir-associated nephrotoxicity at dose levels of 60 or 120 mg once daily (1, 5, 10, 11). In contrast, ADV has shown promise in preliminary clinical trials against chronic HBV infection with wild-type and lamivudine-resistant viruses (6, 13, 15, 16). Indeed, two controlled studies (8) showed that 12 weeks of oral ADV (at 30- and 60-mg-per-day dosages) significantly reduced HBV DNA levels compared to those with a placebo. The HBV seroconversion rate was 20%, and ADV was well tolerated.

HBV resistance to lamivudine occurs in approximately 15 to 32% of both immunocompetent HBV-infected patients and HIV-infected patients after 1 year of lamivudine therapy. The 4-year incidence of HBV lamivudine resistance is 90% in coinfected patients treated with antiretroviral therapy including lamivudine (2). An open-label pilot study evaluated the safety and efficacy of ADV (10 mg once daily) in the treatment of lamivudine-resistant HBV infection in HIV-infected patients. Thirty-five HIV-HBV-coinfected patients receiving lamivudine (150 mg twice a day) as part of their antiretroviral therapy were enrolled in this 12-month study. The results indicated that dosing with 10 mg of ADV daily, in combination with lamivudine, for 7 months is well tolerated and has significant activity against lamivudine-resistant HBV in these HIV-HBV-coinfected patients (2). For most patients, HIV-1 RNA was controlled at screening. Because the dose of 10 mg of ADV once daily was suboptimal for control of HIV infection, we analyzed the HIV-1 reverse transcriptase (RT) genes of patients whose HIV infections were not controlled by antiretroviral drugs during the trial (HIV-1 RNA levels of >400 copies/ml). The aim of this study was to determine if prolonged ADV therapy at a suboptimal dose against HIV-1 replication could select ADV-related mutations at codons 65 and 70 or any other particular HIV-1 RT resistance profile.

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Thirteen patients experiencing virological failure with antiretroviral therapy were analyzed at baseline and at months 3, 6, and 12 of ADV therapy. Genotyping and HIV-1 resistance testing were performed by automated population-based full-sequence analysis (ABI System). The results of genotypic analysis are reported as amino acid changes at positions along the RT gene compared with the wild-type (HXB2) reference sequence.

Table 1 presents all the RT gene substitutions observed during 1 year of the regimen including ADV. All the patients studied harbored HIV-1 strains with the M184V lamivudine-associated resistance mutation at baseline and during all the follow-ups. None of the ADV-associated resistance mutations at RT codons K65R and K70E described previously (4, 7) were observed either at baseline or after 3, 6, or 12 months of ADV treatment. Among the classical RT mutations associated with resistance to nucleoside analogs (9), E44D occurred at month 6 in one patient (patient 7), D67N at month 6 in one patient (patient 7), K70R at month 12 in one patient (patient 4), V118I at month 6 in two patients (patients 7 and 12), L210W at month 6 in one patient (patient 3), and K219R in two patients

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TABLE 1. Mutations in the HIV-1 RT gene and antiviral drug susceptibilities observed for 13 patients with uncontrolled HIV-1 replication treated with 10 mg of ADV for lamivudine-resistant HBV infection

Patient	Time (mo)[a]	HIV-1 RNA copies/ml	Mutation in the HIV-1 RT gene [b]												Other mutations	Nucleoside analogs[c] given with ADV	Adefovir IC_{50}[d] (μM)
			M41	E44	D67	T69	K70	L74	V75	V118	M184	L210	T215	K219			
1	0	4,603			N	D	R				V		V/A	Q		D4T + 3TC	32
	7	257,772			N	D	R				V		V	Q			
	12	187,000			N	D	R				V		V	Q	**V60I**		29
2	0	1,498			N		R				V			Q		AZT + 3TC + ddI	35
	6	1,123			N		R				V			Q	**K64R**		
	12	369			N		R				V			Q	**K64R**		41
3	0	10,183	L		N		R	I			V		Y	E		AZT + 3TC + ABC	40
	3	18,845	L		N		R	I			V		Y	E	**K82R, D110N, H198P, T200A**		
	6	15,213	L		N		R	I			V	W	Y	E	**S166G**		
	12	9,794	L		N		R	I			V	W	Y	E	**S166V**		46
4	0	4,223									V					d4T + 3TC	10
	3	2,712									V				**1142V, S162N**		
	6	6,917									V						
	12	4,052					**R**				V				**T39P**		4.4
5	0	3,635									V					d4T + 3TC	7.8
	3	3,027									V	V		**R**	**135L**		
	6	88,373									V			**R**	**135L**		11
6	0	3,080	L		N			V			V	W	Y			d4T + 3TC	37
	3	1,475	L		N			V			V	W	Y				
	6	1,771	L		N				**I**		V	W	Y		**V90I**		
	12	2,000	L		N				**I**		V	W	Y				43
7	0	17,679	L								V	W	Y			d4T + 3TC	25
	3	22,556	L								V	W	Y		**D186Y**	(d4T→ddI)	
	6	21,891	L	**D**	**N**					**I**	V	W	Y		**K43E**		38
8	0	9,425	L			D					V	W	Y			d4T + 3TC	20
	6	5,870	L			D					V	W	Y	**R**	**1142V, F214L**		22
9	0	1,228									V					d4T + 3TC	6
	3	78,358									V						8
10	0	1,169						V			V					ddI + 3TC	9
	6	<200	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
	12	325						V			V				**T39P, T200A**		11
11	0	81,548	L					V			V	W	Y	Q		d4T + 3TC + ddC	32
	3	45,662	L					V			V	W	Y	Q	**1202V**		
	6	54,258	L					V			V	W	Y		**1202V**		
	12	13,257	L					V			V	W	Y		**H221Y**		23
12	0	13,616	L		N		R				V		F	E		d4T + 3TC	28
	3	9,621	L		N		R			**M**	V		F	E			
	6	7,167	L		N		R			**I**	V		F	E			35
13	0	83	L					V			V		Y	E		ddI + 3TC	20
	3	1,209	L					V			V		Y	N			
	6	3,023	L					V			V		Y	E	**1202V**		18

[a] 0, baseline.
[b] Boldfaced mutations were selected during follow-up. NA, nonamplifiable.
[c] d4T, stavudine; 3TC, lamivudine; AZT, zidovudine; ddI, didanosine; ABC, abacavir; ddC, zalcitabine.
[d] IC_{50}, 50% inhibitory concentration. Values are averages for four independent experiments; average standard errors were <10%.

at months 3 (patient 5) and 6 (patient 8). It should be noted that the majority of patients received a thymidine analog (zidovudine or stavudine) concomitantly with lamivudine and ADV in their antiretroviral regimens. In almost all patients, various polymorphic RT mutations at at least one of the following codons were selected during ADV treatment: codons 35, 39, 42, 43, 44, 60, 64, 82, 90, 110, 118, 142, 162, 166, 186, 198, 200, 202, 214, and 221. The T39P and I202V mutations

were the only changes that were found more than once. TheT39P substitution occurred at month 12 in two patients (patients 4 and 10), and the I202V substitution occurred in two patients at months 3 (patient 11) and 6 (patient 13). The susceptibilities of the viruses to adefovir were measured as previously described by using a molecular clone in which RT was deleted (a gift from C. Boucher) (3). The viruses did not show notable decreases in adefovir susceptibility. This is in accordance with the findings of previous studies that tested viruses in the absence of selection of K65R or K70E mutations in the RT gene (12).

In summary, among the patients studied who had HIV-1 virological failure, the ADV-associated resistance mutations at RT codons 65 and 70 observed in vitro were not observed after 3 to 12 months of ADV at 10 mg once daily. No ADV-specific resistance pattern seems to emerge among RT mutations associated with nucleoside analogs or RT gene polymorphic mutations at this suboptimal concentration of ADV against HIV replication. It should be noted that the methods used for genotypic analysis in this study involve bulk sequencing of PCR products; these methods typically do not detect HIV-1 variants that represent a minor proportion of the viral population. It is also noteworthy that this study was conducted with patients who had previously received antiretroviral therapy and thus harbored HIV-1 RT gene mutations before the use of ADV. These results cannot, therefore, be extrapolated to HIV nucleoside RT inhibitor-naive (NRTI) patients, because all the viruses had NRTI inhibitor resistance mutations at baseline.

In conclusion, our results indicate that prolonged use of ADV at 10 mg per day did not select a particular profile of RT resistance-associated mutations in HIV-1-infected patients with uncontrolled viral replication.

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