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Favorable Interactions between Enfuvirtide and 1-β-D-2,6-Diaminopurine Dioxolane In Vitro

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We evaluated the in vitro anti-human immunodeficiency virus type 1 (HIV-1) interactions between 1-β-D-2,6-diaminopurine dioxolane (DAPD) and enfuvirtide (T-20) against clinical isolates sensitive and resistant to reverse transcriptase and protease inhibitors. Interactions between T-20 and DAPD were synergistic to nearly additive, with combination index values ranging from 0.53 to 1.06 at 95% inhibitory concentrations. These studies suggest that a combination of T-20 and DAPD might be useful in the treatment of antiretroviral drug-experienced patients.

As resistance of human immunodeficiency virus type 1 (HIV-1) to current antiretroviral drugs increases, new antiretroviral regimens are needed, particularly in situations where two or three previous regimens have failed (6).

1-β-D-2,6-diaminopurine dioxolane (DAPD), a prodrug of 1-β-D-dioxolane guanosine, is a nucleoside reverse transcriptase inhibitor active against both X4 and R5 HIV-1 isolates and currently is in clinical trials. Cross-resistance to 1-β-D-dioxolane guanosine is not associated with mutations that confer resistance to zidovudine (ZDV), lamivudine (3TC), or other nucleosides (3, 4, 8). DAPD has synergistic antiviral effects in combination with ZDV, 3TC, or nevirapine (4).

Enfuvirtide (T-20) is a synthetic peptide that inhibits infection by binding to the helical domain of gp41 and disrupting conformational changes required for membrane fusion (7). T-20 has shown clinical anti-HIV activity (9, 10) and has recently been approved for use in the United States. T-20 has also shown synergistic interactions with several other antiretroviral drugs (7, 14, 15). We have evaluated the in vitro interactions between these DAPD and T-20 against several HIV-1 clinical isolates with various degrees of sensitivity to other antiretroviral drugs.

Peripheral blood mononuclear cells from HIV-1-seronegative donors were obtained by Ficoll-Hypaque density gradient centrifugation of heparinized venous blood. After a 3-day phytohemagglutinin (PHA) stimulation, peripheral blood mononuclear cells were resuspended at a concentration of 10^6 cells/ml in RPMI 1640 medium (Sigma) supplemented with 20% heat-inactivated fetal calf serum (Sigma), penicillin (50 U/ml), streptomycin (50 μg/ml), L-glutamine (2 mM), HEPES buffer (10 mM), and 10% interleukin-2, in 24-well tissue culture plates (Becton Dickinson). Drugs were dissolved using dimethyl sulfoxide for DAPD and phosphate-buffered saline for T-20. They were added simultaneously with the HIV-1 inoculum (1,000 to 3,000 50% tissue culture infective doses/10^6 cells) and incubated at 37°C in a humidified 5% CO2 atmosphere.

Multiply diluted fixed-ratio combinations of drugs (ratio of DAPD to T-20 of 1:0.01 to 1:0.03) or single drugs were added to each well. Each condition was tested in duplicate, and each experiment was repeated at least twice. In addition, uninfected drug-treated toxicity controls were maintained at the highest concentration of each agent tested, singly or in combination.

Cell culture supernatant fluids were harvested and analyzed by enzyme-linked immunosorbent assay (Perkin-Elmer, Boston, Mass.) for HIV-1 p24 antigen production on day 4 or day 7 of culture, depending on the day of peak HIV-1 p24 production. Cell proliferation and viability were assessed by trypan blue dye exclusion.

Multiple-drug-effect analysis (1, 2), based on the median-effect principle and the isobologram technique, was used to analyze combined-drug effects. This method involves plotting dose-effect curves for each drug and for multiply diluted fixed-ratio combinations, after which a computerized calculation of a combination index (CI) is derived. A mutually exclusive model was used. Synergy is defined as inhibition greater than the expected additive effect, and antagonism is defined as inhibition less than the expected additive effect. CI values of <1.0 indicate synergism, those of >1.0 indicate antagonism, and those of 1.0 indicate additivity; for convenience, we refer to CI values between 0.9 and 1.1 as nearly additive.

HIV-$1_{(14a\ Pre)}$ was derived from a subject with AIDS before therapy and does not contain drug-associated mutations; HIV-$1_{(14a\ Post)}$ came from the same subject after 26 months of ZDV monotherapy and has ZDV-associated mutations D67N, K70R, T215F, and K219Q. HIV-$1_{(GL\text{-}278)}$ was provided by Victoria Johnson and isolated from an infected patient following 16 weeks of ZDV plus 3TC therapy. It is ZDV and 3TC resistant, with mutations M41L, M184V, and T215Y. $HIV_{(IIIB)}$ is a laboratory-adapted strain. $HIV_{(R5\text{-}18)}$ is a clinical HIV-1 isolate shown to be R-5 by replication in U87 MG-CD4 cell lines expressing CCR5 and the absence of replication in U87 MG-CD4 cell lines expressing CXCR4 and was also non-syncytium-inducing in MT-2 assays. Protease mutations observed were L10V, K20R, M36I, L63P, A71T, V77I, and L90M, and

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TABLE 1. Viruses studied and mean inhibitory concentrations of DAPD and T-20[a]

Virus	Mean EC_{50}		Mutation(s) associated with RTI and PI
	DAPD (μM)	T-20 (nM)	
HIV-$1_{(IIIb)}$ X4	1.93 (±0.57)	7.36 (±1.87)	None
HIV-$1_{(14aPre)}$ X4	2.62 (±0.69)	15.07 (±8.61)	None
HIV-$1_{(14aPost)}$ X4	2.12 (±0.17)	13.50 (±9.19)	D67N, K70R, T215F, K219Q
HIV-$1_{(GL-278)}$ X4	5.48 (±1.94)	7.85 (±4.74)	M41L, M184V, T215Y
HIV-$1_{(R5-18)}$ R5	9.87 (±1.05)	23.75 (±1.77)	PR L10V, K20R, M361, L63P, A71T, V77I, L90M, RT M41L, A98G, M184V, T215Y

[a] Average of two to three experiments (± standard deviation).

reverse transcriptase mutations were M41L, A98G, M184V, and T215Y.

DAPD was provided by Triangle Pharmaceuticals (now Gilead), Durham, N.C., and T-20 was provided by Trimeris, Durham, N.C.

Both T-20 and DAPD had antiviral activity against all isolates tested. As detailed in Table 1, mean 50% effective concentrations were 4.40 μM (±3.02) for DAPD and 13.51 nM (±5.95) for T-20. No cytotoxicity was observed for either drug used singly or in combination.

Combinations of T-20 and DAPD generally showed synergistic to nearly additive interactions against four of the five isolates tested: $HIV_{(IIIB)}$, $HIV_{(14a\ Pre)}$, $HIV_{(GL-278)}$, and $HIV_{(R5-18)}$ (Table 2). Combination indices ranged from 0.85 to 1.13 at a 50% effective concentration (EC_{50}) and 0.53 to 0.91 at EC95 for these four isolates. Interactions ranged from low-level antagonism to near-additivity against the fifth isolate, $HIV_{(14aPost)}$, with CI values ranging from 1.18 at EC_{50} to 1.06 at a 95% effective concentration (EC_{95}).

Designing salvage treatment regimens for patients in whom multiple antiretroviral regimens have failed is difficult because of complex mutational interactions among individual drugs. Agents acting at different steps of the HIV-1 replication cycle, such as entry inhibitors, may help in situations where broad drug resistance is present. However, adding only one new drug to a failing regimen may be insufficient to prevent further development of drug resistance and drug failure. In such situations, novel drug combinations with different resistance patterns are more likely to succeed.

We evaluated interactions between T-20, a fusion inhibitor, and DAPD, a novel nucleoside reverse transcriptase inhibitor. These two compounds, because of their drug resistance profiles, are promising candidates for use in combination salvage therapy. Studying drug interactions in vitro may help predict which drug combinations are likely to be successful clinically. Combinations that show synergy in vitro, such as ZDV and 3TC, have been very useful, whereas combinations that show strong antagonism in vitro resulted in treatment failure (5, 11, 12, 13).

TABLE 2. Interactions between DAPD and T-20 in vitro[a]

Virus	CI for DAPD and T-20 at various inhibitory concentrations			
	EC_{50}	EC_{75}	EC_{90}	EC_{95}
HIV-$1_{(IIIb)}$ X4	1.13 (±0.33)	0.94 (±0.16)	0.80 (±0.07)	0.72 (±0.07)
HIV-$1_{(14aPre)}$ X4	0.85 (±0.10)	0.83 (±0.21)	0.84 (±0.31)	0.91 (±0.30)
HIV-$1_{(14aPost)}$ X4	1.18 (±0.30)	1.13 (±0.26)	1.09 (±0.25)	1.06 (±0.24)
HIV-$1_{(GL-278)}$ X4	0.98 (±0.23)	0.72 (±0.20)	0.53 (±0.18)	0.43 (±0.16)
HIV-$1_{(R5-18)}$ R5	0.87 (±0.19)	0.80 (±0.10)	0.75 (±0.01)	0.72 (±0.03)

[a] CI < 0.9 = synergy, CI 0.9 > 1.1 = near additivity, CI > 1.1 = antagonism. Results are an average of two to three experiments (standard deviation). Concentrations of DAPD used ranged from 0.5 to 32 μM, and those for T-20 ranged from 2.25 to 40.5 nM.

In this study, T-20 and DAPD showed favorable drug interactions at EC_{95}. In patients, drug concentrations at EC_{95} or greater are generally achieved. T-20 and DAPD showed synergy at all concentrations tested against the R5 isolate with broad resistance to other drugs. Very low-level antagonism to near-additivity was seen at low inhibitory concentrations against one X4 clinical isolate resistant to ZDV, $HIV_{(14a\ Post)}$. This was not the case for the other X4 ZDV- and 3TC-resistant isolate, for which the interactions of T-20 and DAPD were mostly synergistic. The reasons for differences among isolates are not clear, and it is unlikely that the low-level antagonism observed against one isolate at low drug concentrations is clinically meaningful.

Our study supports evaluation of this combination in clinical studies, particularly in antiretroviral drug-experienced individuals. As new antiretroviral drugs emerge, it will be important to combine them judiciously to prevent emergence of resistance and preserve future treatment options.

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