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Stampidine Is a Potent Inhibitor of Zidovudine- and Nucleoside Analog Reverse Transcriptase Inhibitor-Resistant Primary Clinical Human Immunodeficiency Virus Type 1 Isolates with Thymidine Analog Mutations

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We report the antiretroviral activity of stavudine-5'-(*p*-bromophenyl methoxyalaninyl phosphate) (stampidine [STAMP]), a novel aryl phosphate derivative of stavudine, against primary clinical human immunodeficiency virus type 1 (HIV-1) isolates. STAMP inhibited each one of nine clinical HIV-1 isolates of non-B envelope subtype and 20 genotypically and phenotypically nucleoside analog reverse transcriptase inhibitor-resistant HIV-1 isolates at subnanomolar to low-nanomolar concentrations.

Stavudine (STV) is a pyrimidine nucleoside analogue used in the treatment of human immunodeficiency virus type 1 (HIV-1) infection. The rate-limiting step for the generation of the bioactive STV metabolite STV-triphosphate is the conversion of STV to its monophosphate derivative (1–3, 8–10). In an attempt to overcome the dependence of STV on intracellular nucleoside kinase activation, we prepared STV-5'-(*p*-bromophenyl methoxyalaninyl phosphate) (stampidine [STAMP]), a novel aryl phosphate derivative of STV, and structurally similar derivatives of STV, zidovudine (ZDV), and lamivudine (19). The presence of a single *para*-bromine group in the phenyl moiety of STAMP contributes to its unique ability to undergo rapid hydrolysis, yielding the key active metabolite alanyl-STV-monophosphate (4, 14, 17, 18). In preliminary studies, we found that STAMP is substantially more potent than STV in inhibiting the replication of the laboratory HIV-1 strain HTLV_{IIIB} in thymidine kinase-deficient T cells (19). Balzarini et al. prepared similar compounds with the hypothesis that such lipophilic compounds would more easily enter HIV-infected cells, be metabolized in a thymidine kinase-independent fashion, and inhibit HIV replication in thymidine kinase-deficient cells such as macrophages (1–3). The structure-activity relationships that determine the propensity of this class of compounds to undergo hydrolysis and inhibit HIV replication have recently been published (17).

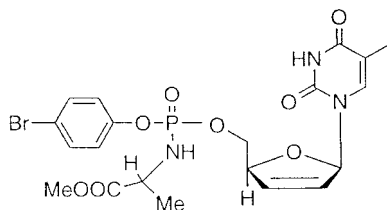
We previously investigated the *in vivo* pharmacokinetics,

metabolism, toxicity, and antiretroviral activity of STAMP in rodent species (4). In mice and rats, STAMP was very well tolerated, without any detectable acute or subacute toxicity at single intraperitoneal or oral bolus dose levels as high as 500

mg/kg of body weight (15). Notably, daily administration of STAMP intraperitoneally or orally for up to 8 consecutive weeks was not associated with any detectable toxicity in mice or rats at cumulative dose levels as high as 6.4 g/kg (15). STAMP exhibited potent *in vivo* anti-HIV activity in human peripheral blood lymphocyte-SCID mice at nontoxic dose levels (16). In accordance with its safety profile in rodent species, a 4-week STAMP treatment course with twice-daily administration to dogs and cats of hard gelatin capsules containing STAMP at 25 to 100 mg/kg was very well tolerated at cumulative dose levels as high as 8.4 g/kg (F. M. Uckun, C. Chen, P. Samuel, S. Pendergrass, T. K. Venkatachalam, B. Waurzyniak, and S. Qazi, submitted for publication). A 4-week treatment course with STAMP administered in gelatin capsules twice daily showed a dose-dependent antiretroviral effect in chronically feline immunodeficiency virus (FIV)-infected cats, as evidenced by a ≥ 1 -log decrease of the FIV load of circulating peripheral blood mononuclear cells (PBMC) within 2 weeks after initiation of STAMP therapy (Uckun et al., submitted).

The purpose of the present study was to evaluate the antiretroviral activity of STAMP against primary clinical HIV-1 isolates. Phenotypic drug susceptibility studies of HIV-1 isolates and strains were performed by measuring the production of the p24 gag protein in PBMC from seronegative healthy volunteers in the presence of increasing concentrations of the

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TABLE 1. Activity of STAMP against ZDV-sensitive primary clinical HIV-1 isolates^a

HIV-1 isolate	Catalog no.	HIV-1 subtype		Origin	IC ₅₀ (μM) ^b		
		gag	env		STAMP	STV	ZDV
92RW016	2061	A	A	Rwanda	0.003	0.1	0.003
92RW008	1746	A	A	Rwanda	0.0008	0.2	0.002
92BR003	1751	B	B	Brazil	0.0006	0.1	ND
92BR025	1777	C	C	Brazil	0.001	0.4	0.004
93IN101	2900		C	India	0.0005	0.1	0.001
93BR019	2314		BF	Brazil	0.0008	0.2	0.002
93BR020	2329	F	F	Brazil	0.007	0.2	0.002
93BR029	2338	F	B	Brazil	0.0008	0.8	0.007
HIV-1 _{JV1083}	3191		G	Nigeria	0.001	0.2	0.003
HIV-1 _{G3}	3187		G	Nigeria	0.0005	0.1	0.01
Mean ± SE					0.002 ± 0.001	0.24 ± 0.07 ^c	0.004 ± 0.001 ^d

^a The primary HIV-1 isolates were obtained through the NIH AIDS Research and Reference Reagent Program.

^b The drug susceptibility assays were performed by measuring the production of the p24 gag protein in PBMC, as described in the methods section. Results are expressed as the average IC₅₀s from two independent experiments, ND, not determined.

^c *P* = 0.0001 (for comparison to STAMP).

^d *P* = 0.04 for comparison to STAMP.

TABLE 2. Activity of STAMP against ZDV-resistant primary clinical HIV-1 isolates

HIV-1 ^b isolate	RT gene mutations	IC ₅₀ (μM) ^a	
		ZDV	STAMP
RT-MDR	M41L, L74V, T215Y, V106N	>10	<0.001
G910-6	M41L, D67N, K70R, T215Y, K219Q	>3.2	0.0028
T156-3	M41L, E44D, D67N, T69D, L210W, T215Y	>3.2	0.0033
G890-1	K20R, M41L, D67N, T69N, K70R, L210W, T215Y	>3.2	0.006
G780-1	M41L, D67N, K70R, T215F, K219Q	>3.2	0.035
C467-4	K20R, D67N, K70R, Y188L, T215F, K219Q	3.2	0.042
G691.2	ND	2.6	0.005
G704-2	M41L, D67N, K70R, L210W, T215Y	1.8	0.0032
P798-1	M41L, T215Y	1.8	0.0075
Q252-2	M41L, L210W, T215Y	1.7	0.0019
Y270-7	M41W, Y188L, T215N	1.5	0.009
S762-4	M41L, T215Y	1.2	0.008
X165-8	D67N, T69N, K70R, K103N, Y181C, T215F, K219E	0.9	0.013
X165-9	K20R, D67N, K103N, T215Y	0.9	0.002
S159-2	M41L, K103N, T215Y	0.7	0.00078
X267-5	M41L, F116S, T215Y	0.6	0.03
X267-1	M41L, L74V, L210W, T215Y	0.6	0.00015
X267-2	M41L, T215Y	0.4	0.00018
R416-10	M41L, T215Y	0.3	0.0002
92BR019	D67N, L214F, T215D, K219Q	0.2	0.002
C140	M41L, M184V, T215Y	0.1	0.0025
Mean ± SE ^c		1.6 ± 0.3	0.0087 ± 0.0027

^a The results presented were obtained from a representative antiviral assay. The SE between individual antiviral assays was <10% of the average IC₅₀.

^b All primary HIV-1 isolates except for 92BR019 were recovered from peripheral blood leukocytes of HIV-infected individuals who had been treated with NRTI using previously described culture techniques (11, 13). RT-MDR is an NRTI-resistant and nonnucleoside analog RT inhibitor-resistant laboratory strain of HIV-1, which was included as a control. HIV-1 RT-MDR-1/MT-2 (catalog no. 252) and 92BR019 (catalog no. 1778; envelope subtype B) were obtained through the NIH AIDS Research and Reference Reagent Program. The drug susceptibility assays were performed using PBMC, as described in the methods section.

^c *P* < 0.0001 (for comparison between ZDV and STAMP; paired *t* test).

anti-HIV agent using the quantitative Coulter HIV-1 p24 antigen enzyme immunoassay and HIV-1 p24 antigen kinetic standard (Beckman Coulter), as previously described (6, 13). We used StatView in the calculation of the 50% inhibitory concentrations (IC_{50} s) from each set of triplicate wells using the linearized form of an exponential equation ($\ln y = \ln b_0 + b_1x$; where y is percent inhibition and x is drug concentration). The inhibition constants were \log_{10} transformed to homogenize the variances within each group. Paired t tests were performed in order to test for differences between means of IC_{50} s for STAMP and STV or ZDV across each viral strain. P values below 0.05 were deemed significant (JMP software; SAS).

Currently available anti-HIV agents have been traditionally developed against subtype B HIV-1 strains, which are the predominant strains in the United States and Europe although worldwide the majority of HIV-infected individuals are infected with non-subtype B strains and the vast majority of new infections are caused by non-subtype B HIV-1 strains (7, 11). Therefore, there is an urgent need to identify anti-HIV agents with potent activity against non-subtype B HIV-1. STAMP (mean $IC_{50} \pm$ standard error [SE] = 1.7 ± 0.7 nM) was 100-fold more potent than STV ($IC_{50} = 240 \pm 7$ nM; $P < 0.0001$ [paired t test on \log_{10} -transformed values]) and 2-fold more potent than ZDV ($IC_{50} = 3.8 \pm 0.1$ nM; $P = 0.039$ [paired t test on \log_{10} -transformed values]) against clinical HIV-1 isolates ($n = 9$) of non-B envelope subtype (viz., subtypes A [$n = 2$], C [$n = 2$], F [$n = 3$], and G [$n = 2$]) originating from South America, Asia, and sub-Saharan Africa (Table 1).

Contemporary antiretroviral treatment regimens employing combinations of drugs from at least two of the three classes of antiretroviral therapy—namely, nucleoside analog reverse transcriptase (RT) inhibitors (NRTI), nonnucleoside analog RT inhibitors, and protease inhibitors—exhibit a potent and sustained antiviral effect and confer consistent long-term viral suppression in patients with HIV infection (12). However, the individual components of these combination regimens can select for drug-resistant viruses, and the emergence of antiviral drug resistance limits the clinical benefit of these drugs (11). Patients failing on contemporary antiretroviral therapy programs constitute a reservoir of multidrug-resistant HIV that may limit treatment options in the future. The frequency of genotypic and phenotypic drug-resistant HIV is increasing among therapy-naïve HIV-infected seroconverters (5). Therefore, there also is an urgent need for new anti-HIV agents capable of inhibiting the replication of NRTI-resistant HIV. STAMP inhibited the *in vitro* replication of each one of 20 genotypically and phenotypically NRTI-resistant HIV-1 isolates carrying two to five thymidine analogue mutations associated with NRTI resistance at nanomolar concentrations, with an IC_{50} (mean \pm SE) of 8.7 ± 2.7 nM, whereas the IC_{50} (mean \pm SE) of ZDV against the same isolates was 1.6 ± 0.3 μ M (t value, 18.1, $P < 0.0001$ [paired t test on \log_{10} -transformed values]) (Table 2). Notably, the phenotypically highly ZDV-resistant G190-6 and G704-2 isolates (ZDV $IC_{50} > 10$ μ M) carrying five thymidine analogue mutations were inhibited by STAMP, with average IC_{50} s of 2.8 and 3.2 nM, respectively. These findings provide evidence that STAMP is a highly potent inhibitor of primary clinical HIV-1 isolates with a ge-

notypic and/or phenotypic NRTI-resistant profile. The documented *in vitro* potency of STAMP against primary clinical HIV-1 isolates with genotypic and/or phenotypic NRTI resistance as well as against the non-B envelope subtype, together with a favorable toxicity profile in rodent (15, 16) and nonrodent (Uckun et al., submitted) animal species, and the *in vivo* antiretroviral activity of STAMP in HIV-infected human peripheral blood lymphocyte-SCID mice (16) as well as in FIV-infected cats (Uckun et al., submitted) warrant the further development of this promising new NRTI compound.

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