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Short communication

Debio-025 inhibits HIV-1 by interfering with an early event in the replication cycle

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ABSTRACT

Cyclophilin A is a peptidyl-propyl isomerase that binds the capsid (p24) protein of HIV-1 and facilitates replication. We report a cyclophilin inhibitor, a non-immunosuppressive cyclosporine analogue, Debio-025, that is about 15-times more potent than cyclosporine A and less toxic resulting in a selectivity index of more than 300. It was equally active against virus strains that were resistant toward inhibitors of the viral entry, fusion, or reverse transcription while it was not inhibitory to HIV-2 or SIV_{MAC}. Mechanism of action studies demonstrate that Debio-025 inhibits the HIV-1 replication by interfering with an early stage of the viral replication cycle. Indeed, addition of Debio-025 could be postponed for 2 h before loosing its antiviral activity. The compound proved inactive against mutant viruses that are independent of cyclophilin A binding suggesting Debio-025 targets the cyclophilin A–capsid interaction.

The replication of the human immunodeficiency virus type 1 (HIV-1) can be drastically reduced by combining potent antiviral drugs at multiple viral targets. The introduction of potent combinations of antiviral drugs, referred to as highly active antiretroviral therapy (HAART), is a major breakthrough in the treatment of HIV infections. Up to now, four distinct steps of the viral replication are targeted: (i) the viral entry (ii) the reverse transcription, (iii) the integration of the provirus into the genome of the infected host cell, and (iv) the proteolytic processing of precursor polyproteins by the viral protease (see reference De Clercq (2009) for review). However, HAART is not able to eradicate HIV from patients. Although established anti-HIV treatments are relatively effective they are sometimes poorly tolerated, highlighting the need for further refinement of the existing antiviral drugs and the development of drugs with other mechanisms of antiviral action.

The cyclophilin A (CypA) interaction with HIV-1 Gag appears as an excellent target for anti-HIV-1 therapy. Few inhibitors targeting the CypA–CA interaction have been described in the past like SDZ NIM811 (Billich et al., 1995; Rosenwirth et al., 1994), CsA analogues (Bartz et al., 1995; Franke and Luban, 1996), and FK506 (Briggs et al., 1999; Karpas et al., 1992). Another cyclosporine analogue, Debio-025 (Fig. 1) has been reported to efficiently inhibit HCV replication (Paeshuyse et al., 2006) and HIV (Ptak et al., 2008). The latter study demonstrated potent activity against clinical and multidrug resistant isolates of various subtypes in peripheral blood mononuclear cells. This drug has also been used to hunt for HIV-1 viruses that are CypA independent and has been shown to inhibit the CypA–CA interaction (Chatterji et al., 2005). It also has been demonstrated to increase HIV-1 vector transduction in primary mouse cells (Noser et al., 2006). Based on its mode of action that is different from that of clinically approved anti-HIV drugs, Debio-025 is a good candidate for further development into a new drug to be included in potent drug combination therapy regimens. The objective of this study was to examine the anti-HIV modalities and the stage at which this non-immunosuppressive cyclosporine analogue inhibits the HIV-1 replication.

First, Debio-025 was evaluated for its anti-HIV and anti-SIV activity in Jurkat cell cultures and its potential to inhibit drug-resistant strains (Table 1). Therefore, A72 cells containing LTR-GFP (Jordan et al., 2003, 2001) were infected with wild-type and respective resistant strains in the presence of test compounds. Production of Tat protein owing to infection drives the integrated LTR to produce GFP. Infected cells fluoresced brightly when measured by flow cytometry or fluorescence microscopy providing a direct and quantitative marker for HIV-1 infection in individual live cells. Cells were harvested 5 days after infection and the number of GFP-expressing cells was monitored by flow cytometry. Toxicity of the compounds was assessed from the forward *versus* side scatter dot plots as well as from an MTT-based viability assay. Debio-025 was active against HIV-1 (III_B) with a 50% effective concentration (EC_{50}) of 0.03 μg/ml

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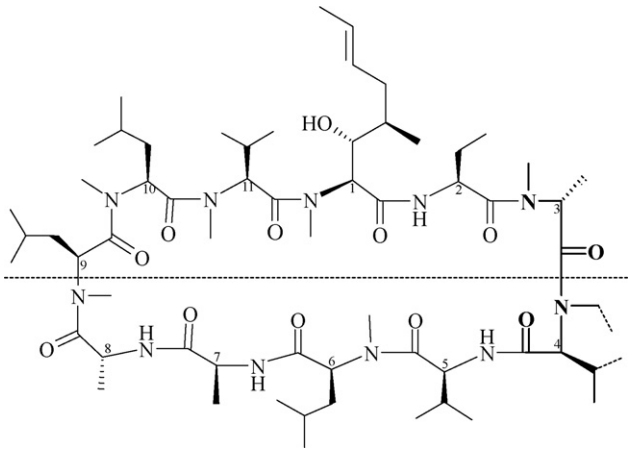

Fig. 1. Structural formula of Debio-025. The line delineates the cyclophilin binding domain (top part of the structure) and the calcineurin binding domain (lower part of the structure).

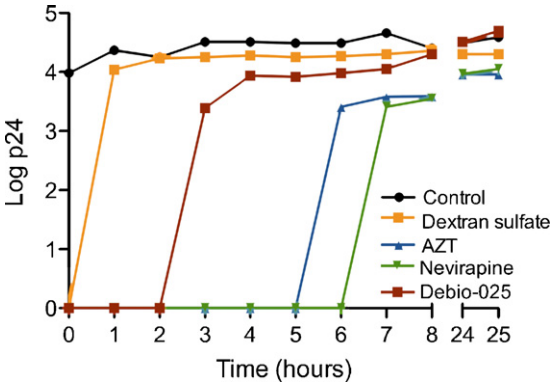

Fig. 2. Time-of-addition experiment. MT-4 cells were infected with HIV-1 (III_B) at a multiplicity of infection of 0.5, and test compounds were added at different times after infection. Virus-associated p24 was monitored at 31 h post-infection as a measure of replication. The data are representative for two separate experiments. Infected but untreated control (no compound); dextran sulfate (100 μg/ml); AZT (0.5 μg/ml); nevirapine (2 μg/ml); Debio-025 (5 μg/ml).

but lacked activity against HIV-2 (ROD). The 50% cytotoxic concentration of Debio-025 was >10 μg/ml resulting in a selectivity index of more than 300. Similarly, CsA was active at subtoxic concentrations against HIV-1 (III_B) and also did not inhibit the replication of HIV-2 lab strains. However, the selectivity index of CsA was limited to 15. Debio-025 was about 15-fold more active against HIV-1 replication.

To assess the potential against drug-resistant strains, the antiretroviral activity of the Debio-025 was tested against strains that are resistant to either the entry inhibitor dextran sulfate (mutations in gp120: S113N, S134N, K269E, Q278H, N293D, N323S, D364-368FNSTW, R387I; 165-fold resistant), the fusion inhibitor T20 (mutations in gp120: A299A/T, D364-368FNSTW; mutations in gp41: L33S, N43K; >17-fold resistant) the NRTI AZT (mutation in RT: A62V, S68G, V75I, F77L, F116Y, Q151M; 194-fold resistant), or the NNRTI nevirapine (mutations in RT: K103N, Y181C; >85-fold resistant) (Table 1). Debio-025 retained its activity against these drug-resistant strains whereas dextran sulfate, T20, AZT and nevirapine were inactive against their respective resistant HIV-1 mutants.

It has been reported that inhibition of CypA-CA interactions inhibits the HIV-1 infectivity, with the block occurring early, at the time of viral DNA accumulation (Berthoux et al., 2004). CypA knock-down also inhibits HIV-1 infectivity with the block occurring early at the time of viral cDNA accumulation (Franke et al., 1994). Therefore, to pinpoint which target along the HIV replicative cycle is affected by Debio-025 we set up a time-of-drug-addition experiment (Fig. 2) (Pauwels et al., 1990). This experiment determines how long the addition of an inhibitor can be postponed before losing its antiviral activity. Indeed, when an inhibitor that interferes with the binding of the virus to the host cell is present at the time of virus binding, it will inhibit the virus replication. However, when the inhibitor is added after virus has already bound to the host cell it will no longer interfere with the viral replication. Therefore, cells were infected at high multiplicity of infection and the compounds were added at 1, 2, 3, . . ., 8 h after infection as indicated. Virus replication was monitored by the assessment of the p24 capsid expression at 31 h after infection. Depending on the target of drug action, addition of the compounds could be delayed for a certain number of hours characteristic for each compound without concomitant loss of antiviral activity. Dextran sulfate, which interferes with the virus adsorption (Baba et al., 1988; Mitsuya et al., 1988), must be added together with the virus (=0 h) to be active; addition at 1 h or later post-infection does not lead to a block of the viral replication because adsorption had already occurred at this time. For AZT the addition could be delayed for about 5 h, while addition of nevirapine could be postponed for 6 h. Addition of Debio-025 can only be postponed for 2 h suggesting a target of interaction happening later than viral adsorption or fusion but before the reverse transcription process, corresponding with a stage at which CypA is known to be essential.

Debio-025 is a non-immunosuppressive cyclosporine analogue; cyclosporines interact with CypA and inhibit the binding of CypA with the p24 capsid protein of HIV-1. For this interaction the loop between position 85 and 99 of the capsid is important. Mutations in this region render HIV-1 CypA independent or resistant to cyclosporines. To determine in more detail whether the inhibition of HIV-1 replication by Debio-025 involves the CypA interaction with HIV-1 CA, we examined the effect of the compound on the infection of a mutant that is independent of CypA binding. HIV-1 CA mutation G89V prevents interaction with CypA (Yoo et al., 1997). C8166 cells were infected with VSV-G pseudotyped HIV containing either the wild-type CA or the mutant G89V CA (a kind gift of Dr. J. Luban) and treated with respective compounds. Infection by G89V CA mutant was about 10-times less than by wild-type virus. The cytotoxicity of the compounds was moni-

Table 1
Antiretroviral activity and cytotoxicity of Debio-025 and CsA in the anti-HIV. GFP-assay in Jurkat cells.

Compound	EC_{50} (μg/ml)								CC_{50} (μg/ml)[a]
	HIV-1						HIV-2	SIV	
	III_B	NL4.3	DS^{res} (165)	$T20^{res}$ (>17)	$NRTI^{res}$ (194)	$NNRTI^{res}$ (>85)	ROD	MAC251	
Debio-025	0.03 ± 0.001	0.04 ± 0.02	0.18 ± 0.09	0.04 ± 0.003	0.02 ± 0.005	0.05 ± 0.04	>10	>10	>10
CsA	0.39 ± 0.16	0.81 ± 0.13	2.63 ± 1.8	0.44 ± 0.07	0.36 ± 0.01	0.31 ± 0.04	>6.19	ND	6.19 ± 2.12
Nevirapine	0.014 ± 0.006	ND	ND	ND	ND	ND	>4	>4	>4

Results are expressed as mean ± S.D. Fold resistance towards the respective inhibitor of resistant strains is given in parenthesis (EC_{50} for III_B strain as 1).

[a] Toxicity was determined in parallel using the MTT-based assay.

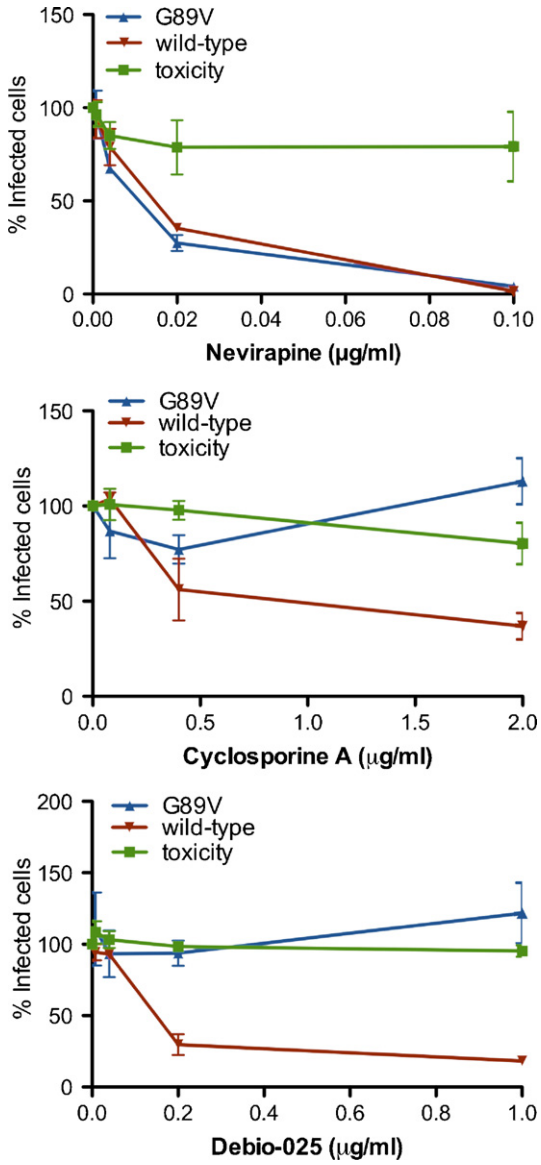

Fig. 3. Debio-025 does not inhibit the replication of the HIV-1 G89V mutant. Wild-type HIV-1 treated with nevirapine (EC_{50}: 0.012 μg/ml), CsA (EC_{50}: 0.67 μg/ml), or Debio-025 (EC_{50}: 0.12 μg/ml) or mutant HIV-1 G89V treated with nevirapine (EC_{50}: 0.008 μg/ml), CsA (EC_{50}: >20 μg/ml), or Debio-025 (EC_{50}: >1 μg/ml) at a range of concentrations as indicated. Infection was assayed for GFP expression at 2 days post-infection. One hundred percent of GFP expression was defined for the infection control (no compound). For the determination of cytotoxicity, uninfected controls were analyzed by an MTT-based viability assay. Results are mean ± S.D. of two independent experiments.

tored in parallel by measuring the viability of mock-infected cells. As expected, nevirapine was active against both the wild-type and the mutant G89V CA HIV-1 viruses. Debio-025 and CsA were able to inhibit the infection of the wild-type HIV-1 while they were inactive against mutant G89V CA HIV-1 (Fig. 3), suggesting the primary target responsible for the antiretroviral activity to be the CypA-CA interaction. At subtoxic concentrations, we observed a stimulation of the mutant G89V CA HIV-1 by Debio-025 and CsA. Future research will validate the significance of this effect.

In conclusion, our results independently confirm the anti-HIV activity of Debio-025 as reported by Ptak et al. (2008). In addition, mechanism of action studies suggest this compound interferes with the CypA–p24CA interaction. Using Debio-025 we could show in our time-of-addition experiment that CypA is important for successful HIV-1 replication during the first 2 h of infection. The CypA-CA interface appears an effective target for development of new anti-HIV therapeutics. No cross-resistance is expected with the clinically used drugs. Therefore, more efforts could be undertaken to elaborate this mechanism as antiviral target. This lead compound and the study of its mechanism of action are useful for the development of future generation of CypA-CA inhibitors with improved activity. Next to its anti-HIV activity, Debio-025 also displays strong anti-HCV activity (Paeshuyse et al., 2006), has a favourable pharmacokinetic and toxicity profile and is in phase II clinical trial for anti-HCV treatment; the compound may prove to be an interesting candidate drug for the treatment of HIV infections, particularly in HIV-1/HCV co-infected patients.

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