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In vitro antiviral activity of 141W94 (VX-478) in combination with other antiretroviral agents

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Abstract

141W94 (VX-478) is a novel HIV-1 protease inhibitor with an IC_{50} of 0.08 μM against HIV-1 (strain IIIB) and a mean IC_{50} of 0.012 μM against six HIV clinical isolates. 141W94 was synergistic on the basis of isobologram analysis with each of the following reverse transcriptase inhibitors: AZT, 935U83, 524W91, 1592U89 and ddI. 141W94 was also synergistic with saquinavir and additive with either indinavir or ritonavir. Resistance to 141W94 has been reported in vitro passage experiments. The binding of 141W94 to human α_1-acid glycoprotein was relatively weak (Kd = 4 μM) and the off-rate for the drug is very fast ($\geq 100\ s^{-1}$). Only a 2-fold reduction of in vitro antiviral activity was observed in the presence of 45% human plasma. No serious drug associated adverse experiences were reported in a Phase I placebo-controlled, single-dose escalation, pharmacokinetic and safety study. The average concentration of 1 41W94 at 8 and 12 h after single doses of 900 and 1200 mg, respectively, was in excess of 10 times the IC_{50}. As 141W94 is synergistic with a variety of anti-HIV-1 agents and exhibits a unique cross resistance profile compared to other protease inhibitors, 141W94 is considered a good candidate for combination therapy.

Keywords: HIV-1; Protease inhibitors; 141W94 (VX-478)

To date, the only antiretroviral drugs approved for the treatment of HIV-1 disease are reverse transcriptase (RT) inhibitors. However, the clinical benefit of these agents as monotherapy is time-limited, in part due to incomplete suppression of virus replication and to the development of resistance. In an attempt to improve on available treatments, antiretroviral agents directed at the HIV-1-encoded aspartyl protease enzyme have been evaluated. Initial in vivo data suggests that these agents can be potent inhibitors of HIV-1, but the durability of their antiviral effect may also be limited due to the development of mutations in the HIV-1 protease coding region. Preliminary in vitro and in vivo data suggest that inhibitors of HIV-1 protease can be safely administered in combination with selected inhibitors of RT and that the combined antiviral effects can be additive or synergistic.

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The HIV-1 protease inhibitor 141W94 (VX-478) was designed using knowledge of the HIV-1 protease crystal structure (Kim et al., 1995). This inhibitor is compact, water soluble, and has demonstrated anti-HIV activity in vitro. The in vitro antiretroviral activity of 141W94 has been studied in both the human T-cell lymphotropic virus type 1-transformed cell line, MT4, and Ficoll-separated, PHA-stimulated peripheral blood lymphocytes (PBLs). 141W94 has been shown to be a potent inhibitor of HIV-1 (strain IIIB) and HIV-2 (strain ZY) with IC_{50} values of 0.08 μM and 0.34 μM, respectively. 141W94 was not toxic to MT4 cells at concentrations as high as 100 μM (the highest concentration tested in this system).

The activity of 141W94 against clinical isolates of HIV-1 was determined. These data provide a comparison to laboratory adapted strains and allow for an assessment of the natural variation in sensitivity to 141W94, and reflect what might be seen in the clinic. PBLs from a geographically diverse set of patients infected with HIV-1 were cocultured with PHA-stimulated donor PBLs. Recovered virus was assayed in PHA stimulated PBLs from normal donors to determine the sensitivity to 141W94. The mean IC_{50} for 141W94 against six AZT-sensitive isolates was 0.012 μM and the mean IC_{50} for 141W94 against three AZT-resistant isolates was 0.019 μM.

In order to explore the ability of 141W94 to prevent the production of infectious virions, the chronically infected, HIV-1 (strain IIIB)-producing cell line, H9IIIB, was exposed to various concentrations of 141W94 for 5 days. At this time, the virus was harvested and used to infect MT4 cells in the absence of compound. The cells were analyzed for cytopathic effect and an IC_{50} of 0.41 $\pm$ 0.08 μM was calculated. In contrast, AZT does not inhibit the production of infectious virions in chronically infected cells.

The antiviral activity of 141W94 in combination with other antiretroviral agents has been determined in MT4 cells acutely infected with HIV-1 (strain IIIB). 141W94 was found to be synergistic with the nucleoside HIV-1 RT inhibitors 935U83, 524W91, 1592U89 or ddI on the basis of isobologram analysis (Fig. 1). 141W94 was also synergistic with the HIV-1 protease inhibitor saquinavir and additive with either indinavir or ritonavir.

Resistance to 141W94 has been generated in in vitro passage experiments (Partaledis et al., 1995). The key resistance mutation in the HIV-1 protease substrate binding site is $Ileu_{50}Val$, which confers approximately 3-fold decrease in susceptibility. This is followed by two additional mutations, also in the substrate binding site, $Met_{46}Ileu$ or $Met_{46}Leu$ and $Ileu_{47}Val$. The virus containing all three protease mutations is about 20-fold less sensitive to 141W94. Comparative work with other protease inhibitors has shown that this resistance profile is unique. Variants resistant to 141W94 have demonstrated limited cross-resistance with saquinavir, indinavir or ritonavir. Virus passaged in the presence of two protease inhibitors has demonstrated a delay in the development of resistance to one or both inhibitors.

The failure of at least one HIV-1 protease inhibitor to achieve adequate antiretroviral effect in vivo has been attributed to binding of the protease inhibitor to human plasma proteins, particularly α_1-acid glycoprotein. (Fischl et al., 1995; Danner et al., 1993; Bilello et al., 1995). Using equilibrium dialysis to determine the bound and free 141W94 concentration in the presence of 2.25, 4.5, and 9.0 μM α_1-acid glycoprotein, the mean K_d value was 3.3 $\pm$ 0.9 μM (Livingston et

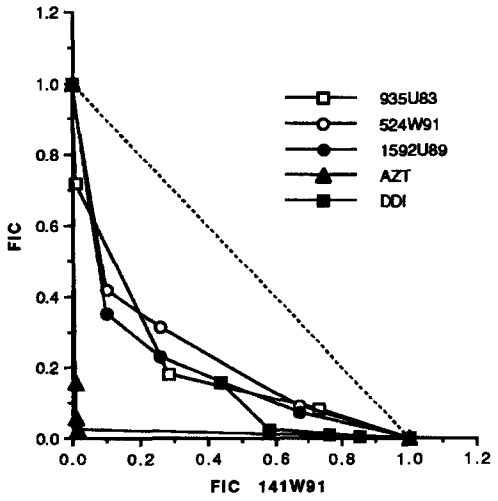

Fig. 1. Fractional inhibitory concentrations.

Table 1
Pharmacokinetics of 141W94

Parameter (units)	Dose				
	150 mg	300 mg	600 mg	900 mg	1200 mg
C_{max} (ng/ml)	1999	3512	6267	7761	9111
t_{max} (h)	1.1	1.3	1.6	1.9	2.1
AUC (O-inf) (h × ng/ml)	4032	9125	21164	32733	47140
$t_{1/2}$ (h)	8.0	7.1	8.0	7.8	9.5
C_8 (ng/ml)	31	135	349	614	1029
C_{12} (ng/ml)	23	91	285	515	641

IC_{50} for laboratory strain = 0.08 μM (40 ng/ml).IC_{50} for clinical isolates = 0.01 μM (6 ng/ml).

al., 1995). In a second, independent experiment measuring the perturbation of the absorbance spectrum of α_1-acid glycoprotein in the presence of 141W94, the K_d value was calculated to be 4 $\pm$ 1 μM. The K_d values determined by two separate methods suggest a weak binding interaction of 141W94 with α_1-acid glycoprotein. Using the determined Kd value of 4 × 10^{-6} M, the estimated off-rate constant for dissociation of 141W94 from α_1-acid glycoprotein is 40–400 s^{-1}, corresponding to a half-time of only 1.7–17 ms.

At 2 μM total drug concentration, 141W94 is approximately 90% bound to human plasma proteins. This percentage is similar to that observed with other protease inhibitors, such as ritonavir, which have exhibited in vivo antiviral activity. The anti-HIV-1 activity of 141W94 was modestly reduced (1.4–2.0-fold) in the presence of 45% human plasma or serum (Livingston et al., 1995). Additional purified human α_1-acid glycoprotein was added to the medium to approximate the actual concentration of α_1-acid glycoprotein found in normal human plasma (18 μM) or plasma from AIDS patients (27 μM). A decrease in anti-HIV-1 activity of only 3–5-fold was observed under these conditions, leading to the conclusion that plasma protein binding should not significantly affect the antiviral efficacy of 141W94 in the clinic.

A Phase I, placebo-controlled, single-dose escalation, pharmacokinetic and safety study was conducted in which oral doses of 150, 300, 600, 900 and 1200 mg of 141W94 were administered to HIV-1 infected volunteers. Serial plasma and urine samples were obtained before and up to 24 h after each dose. A sensitive and specific HPLC method was developed to measure 141W94 in these clinical samples. No serious drug associated adverse experiences were reported in the Phase I study. The average concentration of 141W94 was greater than 10-fold higher than the IC_{50} for the laboratory strain and about 80-fold higher than the IC_{50} for clinical isolates at 8 and 12 h after dosing with the 900 and 1200 mg doses, respectively (Table 1).

141W94 is a potent inhibitor of HIV-1 and HIV-2 in vitro, being active in several cells lines and with both AZT-sensitive and AZT-resistant clinical isolates. Upon passage of HIV-1 in the presence of 141W94, a resistance pattern was generated which is reported to be unique among protease inhibitors. 141W94 was synergistic in vitro with AZT, 935U83, 524W91, 1592U89 and ddI. It also combined favorably with indinavir and ritonavir. While extensive binding to human serum proteins has been observed, the binding of 141W94 to human α_1-acid glycoprotein is relatively weak and the off-rate for the drug is very fast. The in vitro activity of the drug was not greatly affected by the presence of 45% human serum. A Phase I study has demonstrated that 141W94 is safe when administered to HIV-infected individuals at single doses of up to 1200 mg and provides levels of drug greater than the IC_{50} for up to 12 h after dosing. 141W94 is considered a good candidate for further in vivo evaluation and as part of antiretroviral combination chemotherapy regimens.

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