

An IQ Assessment of RDEA806, A Potent NNRTI with an Excellent Activity Profile in the Presence of Human Serum Proteins

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Abstract

Background: RDEA806 is a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) being developed for the treatment of HIV-1 infection. Preclinical testing has shown a better resistance profile of RDEA806 over current NNRTIs against multiple HIV RT mutations, including the most common mutation K103N and the double mutation K103N/L100I. Many NNRTIs bind to serum proteins, effectively reducing the amount of drug available for antiviral activity. To predict a therapeutically effective concentration of RDEA806, *in vitro* antiviral activity was measured in the presence of human serum or serum proteins.

Methods: The antiviral activities of RDEA806 and other NNRTIs were determined using VSV-g pseudotyped HIV-1 containing wild-type and NNRTI-resistant RT sequences in the absence or presence of human serum, human serum albumin (HSA) or α 1 acid glycoprotein (AAG). The EC_{50} values were determined by nonlinear regression analysis, and the EC_{50} shift in the presence of serum proteins was compared to that of other NNRTIs. The RDEA806 serum-adjusted EC_{50} values were compared with the mean C_t and C_{max} values from individuals receiving 400 mg q12h RDEA806 to evaluate the likelihood of achieving effective concentrations of RDEA806.

Results: The fold change (FC) in the EC_{50} value of RDEA806 against wild-type (wt) HIV-1 in 50% human serum is 14, and is lower than the 40 FC observed for efavirenz, 53 FC for etravirine and 161 FC for TMC278. The EC_{50} of RDEA806 shifts 11-fold in the presence of physiologic concentrations of HSA and 1.3-fold in the presence of AAG. RDEA806 also compares favorably with other NNRTIs in the presence of human serum against the most prevalent NNRTI-resistant mutation, K103N. The EC_{50} of RDEA806 against K103N in 50% human serum is 53 nM, compared with 134 nM and 40 nM for efavirenz and TMC278, respectively. After 10 days of q12h 400 mg RDEA806, the mean C_{max} of 5,560 ng/ml and C_t of 279 ng/ml are well above the 22 ng/ml serum-adjusted RDEA806 EC_{50} value against wt HIV-1.

Conclusions: The RDEA806 anti-HIV-1 activity is reduced in the presence of human serum proteins, an effect due predominantly to HSA. The antiviral activity of RDEA806 is less affected by human serum proteins than that of efavirenz, etravirine and TMC278 against a prevalent NNRTI-resistant virus. The RDEA806 inhibitory quotient, the ratio of plasma level to serum-adjusted EC_{50} , is 10-252, suggesting that therapeutically effective concentrations of RDEA806 are achieved.

Introduction

Standard HIV therapies consist of combinations of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs). Treatment failures often occur when viruses that are resistant to one or more components of the regimen arise. Compared to the large numbers of drugs in the NRTI and PI classes, the NNRTI class has only two drugs (efavirenz and nevirapine) in extensive use. There are two important issues that impact the continued use of efavirenz or nevirapine and call for newer drugs in this class: (1) there is a low genetic barrier against resistance development and (2) cross-resistance among approved NNRTIs caused by single amino acid changes, such as the K103N mutation. Transmission of NNRTI-resistant viruses to treatment naïve individuals is also a growing concern. For the treatment of HIV/AIDS, trough levels that exceed the EC_{50} are necessary for therapeutic benefit. RDEA806 is an NNRTI active against many efavirenz and nevirapine resistant viruses, has a high genetic barrier to resistance, and achieves trough levels in humans at least 10-fold higher than the serum-adjusted EC_{50} .

Methods

The antiviral activities of RDEA806 and other NNRTIs were determined using VSV-g pseudotyped HIV-1 containing wild-type and NNRTI-resistant RT sequences in the absence or presence of human serum, human serum albumin (HSA) or α 1 acid glycoprotein (AAG). The EC_{50} and EC_{90} values were determined by nonlinear regression analysis, and the shifts in the presence of serum proteins were compared to that of other NNRTIs. The RDEA806 serum-adjusted EC_{50} values were compared with the mean C_t and C_{max} values from individuals receiving 400 mg q12h RDEA806 to evaluate the likelihood of achieving effective concentrations of RDEA806.

Table 1. Protein Binding in Media Containing 2% FBS

Compound	% Bound in Media	% Bound in Media + 40% HS
RDEA806	86.6 ± 0.08	99.3 ± 0.01
TMC278	71.7 ± 1.17	99.6 ± 0.01

Radio-labeled RDEA806 or TMC278 were incubated in media containing 2% FBS or media containing 2% FBS and 40% human serum (HS). Unbound compound was recovered after pelleting protein by ultracentrifugation.

- Both NNRTIs are highly bound by serum proteins
- The relatively higher protein binding by RDEA806 in media may account for its lower potency compared with TMC278, as their potencies are similar in 40% human serum

Table 2. Protein-Adjusted Activity of NNRTIs Against HIV-1

Compounds	HIV-1 wild type				
	2% FBS ^a	+ HSA ^b	+ AAG ^c	+ AAG+HSA	+40% HS ^d
RDEA806	5.1	68	6.1	41	51
Etravirine	0.61	2.3	3.4	2.9	24
TMC278	0.66	28	0.61	16	38
Efavirenz	0.40	9.3	1.1	6.5	6.6

^aAll *in vitro* EC_{50} determinations performed in 2% FBS-media in JCSJ cell line

^b1µg 45 ng/ml human serum albumin (HSA)

^c1µg 1 mg/ml α 1 acid glycoprotein (AAG)

^d1µg 40% human serum (HS)

To assess the antiviral efficacy of protein-bound compounds, virus, cells and compounds were tested in 2% FBS media plus individual human serum proteins and physiological concentrations or in 2% FBS media plus 40% human serum.

- The effect of 40% human serum on EC_{50} was greater than either the individual or combined effects of the major serum protein components, HSA and AAG, for etravirine and TMC278
- Antiviral activity determined in the presence of human serum may be a more accurate indicator of clinical activity

Table 3. Activity in Human Serum

Compounds	HIV-1 wild type			
	2% FBS	+40% HS	2% FBS	+40% HS
RDEA806	5.1	51	15	160
Etravirine	0.61	24	2.8	94
TMC278	0.66	38	2.3	122
Efavirenz	0.40	6.6	1.4	30

^aRDEA806 data are the mean values from 12 independent experiments

- RDEA806 has similar potency to etravirine and TMC278 against wild-type HIV-1 in 40% HS although it appears to be much less potent in 2% FBS

Table 4. Excellent Activity Against Double-Mutant Highly-Resistant to EFV

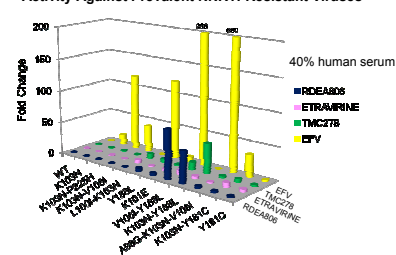
Compounds	HIV-1 L100I-K103N		EC ₅₀ nM	
	2% FBS	+40% HS	2% FBS	+40% HS
RDEA806	2.4	47	11	181
Etravirine	2.2	143	21	811
TMC278	6.8	425	24	1910
Efavirenz	445	>10,000	>2,500	>10,000

^aL100I-K103N present in 10.6% of efavirenz-exposed treatment failures¹

- RDEA806 is more active against the K103N-L100I mutant than the other NNRTIs

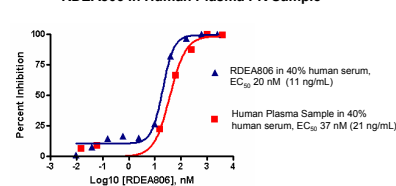
Results

Figure 1. Fold Change in EC_{50} Values of Protein-Adjusted Activity Against Prevalent NNRTI-Resistant Viruses



- RDEA806 has a better resistance profile against prevalent NNRTI-resistant viruses than efavirenz in the presence of 40% human serum
- The activity of RDEA806 is less affected by the Y181C, K103N-Y181C and K103N-L100I mutants than etravirine and TMC278

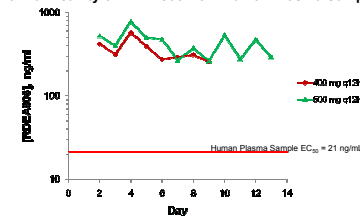
Figure 2. In Vitro Antiviral Activity of RDEA806 in Human Plasma PK Sample



The *in vitro* antiviral activity of RDEA806 in human plasma taken after 10 days of 400 mg dosing was measured in the VSV-g-pseudotyped, single cycle infection system. Activity from a titration of this sample into 40% human serum was compared with activity from a titration of RDEA806 in 40% human serum and is shown in the dose response inhibition curve.

- Circulating levels of RDEA806 in human plasma after 10 days of 400 mg q12h suppressed HIV replication *in vitro* to an extent similar to RDEA806 in assays containing 40% human serum
- The RDEA806 protein-adjusted EC_{50} determined in 40% human serum may be an appropriate estimation of antiviral activity

Figure 3. Comparison of Mean RDEA806 C_t Concentrations to Antiviral Activity of RDEA806 from Human Plasma Samples



Concentrations of RDEA806 in human plasma were determined after q12h dosing with 400 mg Modified Release Capsules, or 500 mg standard capsules of RDEA806. Shown are the mean C_t values from a Phase 1 multiple-ascending dose clinical trial in healthy volunteers.

- RDEA806 levels in plasma at 400 or 500 mg q12h are well above the protein-adjusted EC_{50} values in actual human plasma samples for HIV-1
- The Inhibitory Quotient_{plasma concentration/protein-adjusted EC_{50}} was 13-265

Conclusions

- RDEA806 is highly protein bound in standard 2% FBS media, so that the relative shift in activity in the presence of human serum proteins is less pronounced than that of other NNRTIs.
- The reduction of RDEA806 anti-HIV-1 activity in the presence of human serum proteins appears to be an effect due predominantly to HSA.
- The antiviral activity of RDEA806 is less affected by human serum proteins than that of efavirenz, etravirine and TMC278 against a prevalent NNRTI-resistant virus.
- The *in vitro* antiviral activity of RDEA806 determined from human plasma samples correlates with the *in vitro* values determined in 40% human serum.
- The RDEA806 IQ_{plasma} , the ratio of plasma level to serum-adjusted EC_{50} , is 13-255, suggesting that therapeutically effective concentrations of RDEA806 are achieved. Once and twice daily dosing of RDEA806 is being evaluated in an on-going Phase 2a study in antiretroviral naïve patients.
- In Phase 1, RDEA806 levels in plasma were well above the protein-adjusted EC_{50} and EC_{90} values for HIV-1 at all doses tested. All doses were well tolerated with no serious adverse events, no grade 3 or 4 adverse events and no clinically significant laboratory or ECG abnormalities. Phase 2a is currently enrolling.

References

- Bachelor L.T., ED Anton, P Kudish et al. 2000 *Antimicrob. Agents Chemother.* 44:2475-2485