PREZISTA® (darunavir)
PREZISTA - Mechanism of Action / Binding Affinity

SUMMARY

- In an in vitro study, darunavir (DRV) was shown to inhibit dimerization of HIV-1 protease subunits at very low concentrations while lopinavir (LPV), ritonavir (RTV), indinavir (IDV), saquinavir (SQV), nelfinavir (NFV), amprenavir (APV), and atazanavir (ATV) did not.[1]
- In another study, the binding affinity of DRV to wild-type (WT) protease was more than 2 orders of magnitude higher than that of the other PIs tested, generally due to a high association rate (k_{on}=2.2 x 10^6 $M^{-1}s^{-1}$) and a very low dissociation rate (k_{off}=7.8 x 10^{-7} s^{-1}).[2]
    - Dissociative half-life, which represents the duration of interaction between the drug and the protease, was highly extended with DRV (>240 hours) as compared with APV (13.5 minutes), ATV (1.4 hours), LPV (1.2 hours), and tipranavir (TPV) (1.8 hours).
- In a study conducted with DRV and APV, the binding constant for DRV was 87-fold greater against WT protease and 33-fold greater against a mutant protease than that of APV.[3]

BACKGROUND

The genetic diversification of human immunodeficiency virus (HIV) is attributable to rapid virus replication, yielding 10^{10} new virions per day, and the genetic recombination ability of the virus.[4] Treatment with antiretroviral therapy exerts additional selection pressure because most antiretroviral agents exploit particular features of the virus that, once targeted, inherently encourage development of resistance.[4,5]

HIV protease is a dimeric structure composed of 2 monomers with flaps at the entrance to the active site, which fold over the substrate when binding takes place to seal the site and promote catalysis; the flaps then reopen, allowing release of cleaved products.[4,6] Effective protease inhibitors (PIs) inactivate the protease by causing the flaps to remain tightly closed after binding, duplicating the transitional state of the protease, thereby inactivating the enzyme.[6]

When HIV-1 protease cleaves the substrate, which takes place in diverse but highly specific sequences, an asymmetrical "substrate envelope" is formed, defining a specific region within the active site that is crucial for recognition of the substrate.[6] Structural analysis shows that protease in mutant HIV-1 strains largely maintains the backbone conformation of the WT protease.[4] Any mutation that alters key structural elements such as the backbone conformation would impede viral fitness because of loss of catalytic function. The premise underlying development of DRV was that an inhibitor that acts through hydrogen-bonding interactions at the backbone of protease would be potent against WT as well as mutant strains, since the backbone structure is essential to viability of the virus.[7] The potency of DRV is attributed to its ability to form strong hydrogen bonds at the main chains of aspartic acids in the S2 subsite.[5]

The shapes of protease complexes with most PIs differ considerably from that of the substrate envelope, leaving locations that remain in contact with the protease. Primary drug-resistant mutations often arise at positions in the protease where the inhibitors protrude beyond the substrate envelope but are still in contact with the enzyme.[6] DRV is able to closely mimic substrate interactions because of its flexible conformation, which produces a close fit within the substrate envelope, as well as its mimicry of substrate hydrogen bonds, which contributes to exceptionally tight bonding. These features provide potent antiviral activity and a high barrier to development of resistance.[4,6]

In vitro experiments showed that resistant HIV strains were easily produced in the presence of other PIs, but selection and replication of viable resistant HIV strains was difficult in the

presence of DRV.[8] Furthermore, viruses isolated during exposure to DRV did not readily replicate even when DRV was no longer present, suggesting that mutant variants selected by DRV had a lower replication rate than is typically seen in both WT and other mutant HIV strains.

DIMERIZATION

Dimerization of HIV-1 protease subunits is a critical step for HIV-1 protease to acquire its proteolytic activity.[9,10] Consequently, a PI acting as an HIV-1 dimerization inhibitor in addition to the conventional mechanism of action by which PIs inhibit HIV-1 protease could result in highly potent inhibition of HIV-1.[11]

Koh et al (2007)[1] evaluated the effect of various PIs on protease dimerization using an intermolecular fluorescence resonance energy transfer (FRET)-based HIV-1–expression assay. DRV and TPV were shown to inhibit protease dimerization at very low concentrations (0.1 µM), while LPV, RTV, IDV, SQV, NFV, APV, and ATV did not.

BINDING AFFINITY

Dierynck et al (2007)[2] characterized the binding kinetics of DRV and 8 comparator PIs (APV, ATV, LPV, TPV, IDV, NFV, RTV, and SQV) to WT protease to provide insight into the molecular basis for the high potency of DRV and broad-spectrum activity. Binding kinetics for DRV and 4 of these agents to 5 mutant proteases harboring 10 to 14 IAS-USA (2005) PI resistance-associated mutations (RAMS) were also determined.

Methods

- Surface plasmon resonance was used to determine individual rate constants for association (k_{on}) and dissociation (k_{off}) and a derived affinity constant Kd (k_{off}/k_{on}).
- The dissociative half-life ($t_{1/2}$) was determined from these data.

Results

- The binding of DRV to WT protease (Kd=4.1 x 10^{-13} M; data outside of the detection limit) was high.
- This high affinity is due to the high association rate (k_{on}=2.2 x 10^{6} $M^{-1}s^{-1}$) and a very slow dissociation rate (k_{off}=7.8 x 10^{-7} s^{-1}).
- The binding affinity of DRV to WT protease (K_d <10^{-12} M) was more than 2 orders of magnitude higher than that of the other PIs.
- Dissociative half-life, which represents the duration of interaction between the agent and the protease, was highly extended with DRV (>240 hours) as compared with APV (13.5 minutes), ATV (1.4 hours), LPV (1.2 hours), and TPV (1.8 hours).
- Binding affinity to the mutant proteases was lower for all agents than to WT protease; for DRV, this lowered binding affinity was proportional to the number of DRV RAMs present and primarily attributable to faster dissociation.
- Unlike the other PIs, DRV did not show decreased antiviral activity against the corresponding viral strain when binding affinity to a mutant variant decreased by up to 1000-fold from the binding with WT protease, although a decrease in antiviral activity was seen with decreases of greater than 1000-fold.

King et al (2004)[3] evaluated the structures and binding thermodynamics of DRV and APV in complex with WT and a multi-drug resistant (MDR) variant (L63P, V82T, and I84V) of HIV-1 protease.

Methods

- X-ray crystallographic structures of the complexes were determined.

- Isothermal titration calorimetry was used to establish thermodynamics of binding, with binding affinities determined by the replacement titration method for both the WT and MDR proteases.

Results

- Analysis of the complex between the WT protease and DRV showed that DRV formed shorter, tighter hydrogen bonds than APV.
- In addition, DRV protruded further from the substrate envelope, allowing formation of additional hydrogen bonds not seen with APV.
- Most of the hydrogen bonding interactions with DRV were with the main-chain atoms at the bottom of the active-site cleft.
- Complexes with both agents showed flexibility in proteins and ligands which allowed conformational adjustments that were able to compensate for effects of mutations on binding.
- The hydrogen-bonding pattern of the DRV complex with MDR protease showed few differences from the DRV complex with WT protease.
- Both DRV and APV showed tight binding, with dissociation constants for the WT complex of 4.5×10^{-12} M with DRV and 3.9×10^{-10} M for APV; binding was 87-fold tighter with DRV than with APV.
- Although DRV showed a greater loss of affinity than APV when complexed with mutant protease, DRV binding was nonetheless 33-fold greater than that of APV, and dissociation constants were 6.0×10^{-11} M and 2.0×10^{-9} M, respectively.

LITERATURE SEARCH

A literature search of MEDLINE®, Embase®, BIOSIS Previews®, and Derwent Drug File (and/or other resources, including internal/external databases) pertaining to this topic was conducted on 13 September 2024.

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