

Anti-HIV Activity of Aprepitant and Synergistic Interactions with other Antiretrovirals

Poster Board #: 750

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Abstract

Background: The neuropeptide Substance P is a major mediator of neurogenic inflammation and immunomodulatory activities within the central and peripheral nervous system. Drugs which interfere with binding of Substance P to the neurokinin 1 receptor NK-1, have been shown to reduce the inflammatory response. Aprepitant, an NK-1 receptor antagonist, currently used clinically for controlling nausea and vomiting caused by chemotherapy, had recently been shown to also inhibit HIV infection, in part, through down-regulation of CCR5, a principal co-receptor for HIV entry into macrophages. In this study, we tested additional SP antagonists for potential anti-HIV-1 activity, and investigated potential synergies with other antiretroviral drugs.

Methods: A series of SP Agonists, including Aprepitant, CJ-12255, CJ-96345, RP-67-580, and L733060 were tested for potential in vitro antiviral activity against CCR5-tropic (HIV-1_{BA-L}) or CXCR4-tropic (HIV-1_{INL4}) viral isolates. Viral replication in peripheral blood mononuclear cells from 3 different donors was measured by quantifying p24 production (ELISA). The 50% inhibitory concentration (IC₅₀) was determined for each drug. Synergistic effects of combinations of Aprepitant with representatives of each of the major classes of approved anti-HIV drugs were evaluated for inhibition of HIV-1_{BA-L} in PBMC.

Results: Aprepitant shows the highest antiretroviral activity (IC₅₀ 5.4 μM) of the NK-1R antagonists tested. It also exhibited good efficacy (IC₅₀ 7.95 μM ± 3.88) against primary infection by each major HIV-1 subtype (A, B, C, D, AE, F, G, H and O), including CXCR4- and CCR5-tropic primary isolates propagated in PBMC, with low cytotoxicity > 25 μM. Strong synergistic effect was observed for Aprepitant with Ritonavir, and to a lesser degree with AZT and ddC. No significant synergies were observed with ddI, 3TC, and T-20.

Conclusions: The NK-1R antagonist, Aprepitant, has been shown to have low, but reproducible antiviral effect on a broad range of HIV isolates in PBMC culture. The fact that this drug is already used clinically to control nausea in chemotherapy and has demonstrable anti-HIV activity which is synergistic with other antiretrovirals make it an excellent candidate as a potent anti-HIV therapeutic agent. Phase I clinical trials for use of Aprepitant as an anti-HIV agent are underway.

Introduction

The neuropeptide Substance P is a major mediator of neurogenic inflammation and immunomodulatory activities within the central and peripheral nervous system. Drugs which interfere with binding of Substance P to the neurokinin 1 receptor NK-1, have been shown to reduce the inflammatory response. An NK-1R antagonist Aprepitant (Emed, Merck & Co., Inc.), is approved by the FDA for the prevention of both acute and delayed chemotherapy-induced nausea and vomiting (1). The SP-NK-1R interaction is also important in viral infections, including HIV infection of human immune cells. Earlier studies demonstrated that the non-peptide SP antagonists CP-96,345 (2) and aprepitant (3), downregulate CCR5, a principal co-receptor for HIV entry into macrophages, and inhibits their ability to be infected with HIV in vitro. In this study, we tested additional SP antagonists for potential anti-HIV-1 activity in vitro, including their inhibition of a wide range of HIV subtypes. Additional studies investigated potential in vitro synergies of combination treatments of aprepitant with other antiretroviral drugs.

Materials and Methods

A series of SP Agonists, including Aprepitant, CJ-12255, CJ-96345, RP-67-580, and L733060 were tested for potential in vitro antiviral activity against CCR5-tropic (HIV-1_{BA-L}) or CXCR4-tropic (HIV-1_{INL4}) viral isolates. Viral replication in peripheral blood mononuclear cells from 3 different donors was measured by quantifying p24 production (ELISA). The inhibitory activity of Aprepitant was also tested against a panel of primary HIV-1 isolates grown in PBMC, which included representatives of a broad range of HIV Subtypes, representing both T lymphotropic (CXCR4) and macrophage-tropic (CCR5) primary isolates. Synergistic effects of combinations of Aprepitant with representatives of each of the major classes of approved anti-HIV drugs were evaluated for inhibition of HIV-1_{BA-L} in PBMC.

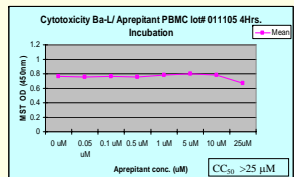
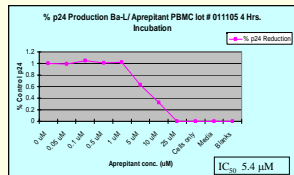
Inhibition of HIV infectivity of PBMC in vitro was measured in 6-well plates containing 200,000 PBMC per well, and decreasing concentrations of drug.

Virus infectivity (TCID50) was measured in duplicate wells by assay of the supernatant harvested on day 7 by the p24 antigen ELISA (Coulter). IC50 was calculated as the concentration of drug which inhibits virus by 50%.

Cytotoxicity (IC50), the drug concentration which causes toxicity in 50% of the cells, was determined by MTS assay on day 4.

Synergy studies were performed in 96-well plates containing 3 x 10⁵ PBMC per well and infected with Bal-1 virus at moi = 0.01. Serial dilutions of Aprepitant and HIV antiviral drugs were added to the wells in checkerboard configuration, and the plates incubated for 5 days at 37°C. Virus infection was determined by XTT/PMS dye endpoint.

Viral Reduction and Cytotoxicity of Aprepitant



Aprepitant inhibits HIV infectivity of PBMC by Bal-1 (CCR5 tropic) at IC₅₀ 5.4 μM and shows very limited cytotoxicity even at 25 μM

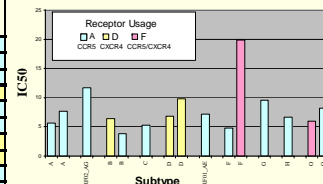
Anti HIV Activity of NK-1R Antagonists

Name	IC50	CC50	Ca+ Block
Aprepitant	5.4	>25	0.1
L-733,060	5.6	>25	3
RP-67,580	11	>25	>100
L-759-274	13.8	>25	0.3
CP-96-345	15.2	>25	1
CP-99,994	No activity	>25	0.01
CJ-12,225	No activity	>25	100

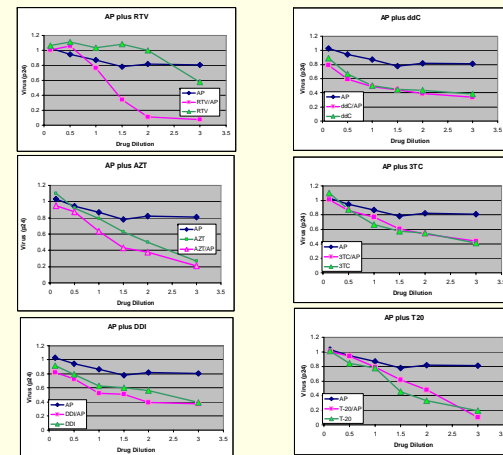
AZT	0.011	>10	N/A
ddC	0.008	>10	N/A

- Several NK-1R Antagonists have anti-HIV activity
- Of these, Aprepitant exhibits the strongest anti-HIV activity with 5.4 μM IC₅₀
- Antiviral activity has no apparent correlation of Ca⁺ Block activity
- All compounds have very low toxicity >25 μM EC₅₀

HIV-1 Subtype	Isolate ID	Country of Origin	NSI/SI	Co-receptor	Aprepitant IC50 (nM)
A	UC275	Uganda	N	R5	5.65
A	I-2496	Ghana	N	R5	7.65
CRF02_AG	DI263	Djibouti	N	R5	11.7
B	BZ167	Brazil	S	X4	6.37
B	US2	USA	N	R5	3.86
C	ZAM18	Zambia	N	R5	5.33
D	SE365	Senegal	S	X4	6.85
D	UC270	Uganda	S	X4	9.82
CRF01_AE	ID17	Indonesia	N	R5	7.13
F	IB163	Brazil	N	R5	4.79
F	BCLR07	Romania	S	X4/R5	19.86
G	1991	Zaire	NSI	R5	9.53
H	BCLKITA	Zaire	N	R5	6.66
O	BGF06	Ghana	S	X4/R5	5.97
O	BGF-11	US	S	R5	8.15



- Aprepitant is effective against all HIV Subtypes examined including Subtypes A-H, CRFs, and O
- Aprepitant is effective against HIV isolates utilizing CCR5, CXCR4, or both receptors in PBMC



- Protease Inhibitor Ritonavir shows very strong synergistic effects with Aprepitant
- Nucleoside Reverse Transcriptase Inhibitors AZT and DDI show moderate, but detectable synergies with Aprepitant
- No measurable synergy was observed with other HIV-1 drugs examined

Conclusion

- Aprepitant showed the highest antiretroviral activity (IC₅₀ 5.4 μM) of the NK-1R antagonists tested, and had low cytotoxicity (CC₅₀ > 25 μM).
- Aprepitant inhibited primary infection in PBMC by each major HIV-1 subtype (A, CRF02_AG, B, C, D, CRF02_AE, F, G, H and O). Isolates which primarily utilize CXCR4 and CCR5 or both co-receptors were inhibited, with no apparent differences based on receptor usage.
- Strong synergistic effects were observed for Aprepitant with the Protease Inhibitor Ritonavir, and to a lesser degree with Nucleoside Reverse Transcriptase Inhibitors AZT and DDI. No significant synergies were observed with ddI, 3TC, and T-20.
- The fact that this drug is already used clinically to control nausea in chemotherapy and has demonstrable anti-HIV activity which is synergistic with other antiretrovirals make it an excellent candidate as a potent anti-HIV therapeutic agent.
- Phase I clinical trials for use of Aprepitant as an anti-HIV agent are underway.

References

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