

Greater Synergistic Anti-HIV Effects upon Combination of a CCR5 inhibitors than with other anti-HIV drugs

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Background

CCR5 represents a major chemokine receptor which R5-HIV exploits in its entry to target cells, thus serving as an attractive target for possible intervention of R5-HIV infection. AK602/ONO4128/GW873140, a novel CCR5 inhibitor containing a unique spirodiketopiperazine group, potently blocks *in vitro* the infectivity of R5 HIV with subnanomolar IC₅₀s and this compound is now under a phase II clinical trial. Here we determined anti-HIV activity upon combination of AK602 with various anti-HIV agents including recently reported entry inhibitors.

Methods

Experimental design

PBMC from buffy coat of HIV-1-seronegative individuals were obtained by Ficoll-Hypaque density gradient centrifugation and were cultured at a concentration of 10⁶ cells/ml in RPMI 1640-based culture medium supplemented with 10% fetal calf serum, penicillin (50 U/ml) and streptomycin (50 μg/ml) ("10% FCS-RPMI") with 10 mg/ml phytohemagglutinin (PHA) for 3 days prior to the anti-HIV-1 activity assay *in vitro*. After 3 days' PHA-stimulation, PBMC (10⁶/ml) were resuspended in 10% FCS-RPMI containing 10 ng/ml interleukin-2 in 96-well microculture plates. Single drug or combinations of drugs were added to each well at serial dilutions. The cells were exposed to R5 HIV-1_{BaL} or a mixtures of R5 HIV-1_{BaL} plus X4 HIV-1_{1049pe} at 50:50 ratio at 100 50% tissue culture infective dose (TCID₅₀s) and were incubated at 37° C in a humidified air containing 5% CO₂. On day 7 cell-free culture supernatants were harvested and HIV-1 p24 antigen levels were determined using a fully automated chemiluminescent enzyme immunoassay system. All the assays were performed in duplicate and each experiment was independently conducted 5-10 times. No cytotoxicity was observed at the highest concentrations of each agent as assessed with the trypan blue dye exclusion method.

Anti-HIV agents

Two reverse transcriptase inhibitors (AZT, NVP), protease inhibitor (IDV), fusion inhibitor (T-20), entry inhibitor (sCD4), two CCR5 inhibitors (TAK-779, Sch-C), and two CXCR4 inhibitors (AMD3100, TE14011) were used.

Viruses

R5-tropic HIV-1_{BaL} was obtained from NIH Reagent Program. X4-tropic HIV-1_{1049pe}, a clinical strain from drug-naïve patient, was also used. HIV-1 strains were passaged once or twice in PHA-PBMC cultures and the culture supernatants were stored at -80° C until use.

Mathematical analysis

The antiviral activity of combined agents was analyzed using the method described by Prichard *et al* (Bliss Independence method). The theoretical additive interactions from the monotherapy groups were determined by the independent equation and were plotted as a plane in a 3-dimensional graph. Then the combination effects were compared with the predicted additive effects. In the original method the plots above the additive plane represent synergistic interaction, while the points below the plane represent antagonism. To investigate the drug interaction further, we determined the average volume of all combination plots in each experiment and we statistically compared the average combination effects between the combinations of two agents and a single agent. Nonparametric statistical analyses were performed by using the Mann-Whitney *u* test. We also assessed the antiviral effects of two compounds treatments using the Combination Index (CI) based on the multiple-drug effect analysis of Chou and Talalay. CIs of <1, =1 or >1 indicates synergistic effect, additive effect and antagonism, respectively.

Results

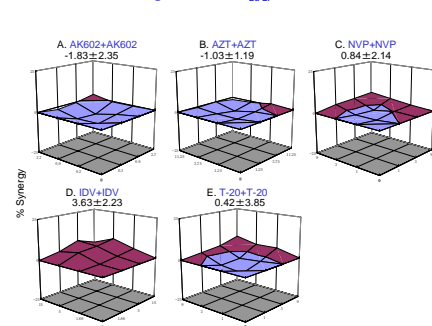
Synergistic effects were seen when AK602 was combined with AZT, NVP, IDV, sCD4, or T-20 against HIV_{BaL} and HIV_{BaL}1049pe. Mild synergism was also observed with AK602 + TAK779. Additivity was seen with AK602 + SCH-C. Most potent synergism was seen when AK602 was combined with AMD3100 or TE14011 against HIV-1_{BaL}1049pe. No antagonistic anti-HIV effects or synergistic toxicity was seen in binary combinations of two agents. Similar results were obtained when we analyzed the data using the median-effect principle and were expressed as combination indices.

Table 1 Antiviral activity in the present assay system

Compound	Anti-HIV-1 activity (μM)	
	IC ₅₀	IC ₉₀
AK602	0.0007 ± 0.0004	0.016 ± 0.015
SCH-C	0.006 ± 0.006	0.094 ± 0.043
AZT	0.018 ± 0.004	0.128 ± 0.054

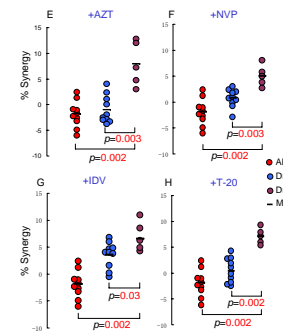
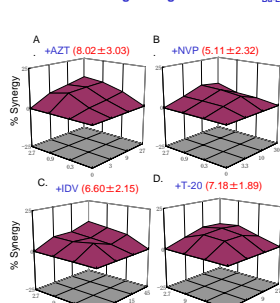
p24 assay (cell: PBMC, virus: HIV_{BaL})

Figure 1 3D-graph of HIV compound monotherapy against R5-HIV-1_{BaL}.



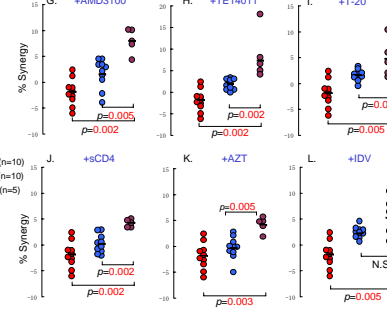
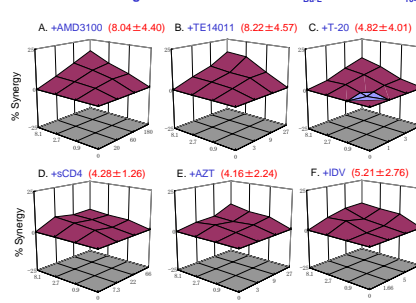
PBMC with anti-HIV compound were cultured with R5-HIV_{BaL} for 7 days and HIV-1 p24 antigen levels were determined. Drug interaction was analyzed with Bliss Independence method and the averages were plotted in 3-D graph (A-E). Averages of all the plotted values were described below the compound's name. Most combination effects of single compound were plotted near the plane, theoretical additive effect, except IDV+IDV. Combination assays using a single compound were performed 10 times. All assays were conducted in duplicate.

Figure 2 Drug interactions between AK602 and other anti-HIV-1 agents against R5-HIV-1_{BaL}.



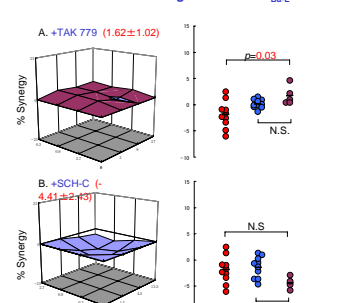
PBMC with combination of drugs at serial dilutions were cultured with R5-HIV_{BaL} for 7 days and HIV-1 p24 antigen levels were determined. Drug interaction analyzed with Bliss Independence method were plotted in 3-D graph (A-D). Then combination effects were statistically compared with monotherapy (E-H). All combination of AK602 plus AZT (E), NVP (F), IDV (G) or T-20 (H) showed synergistic effects. Combination assays of AK602 with other compounds and single compound were performed 5 times and 10 times, respectively. All assays were conducted in duplicate.

Figure 3 Drug interactions with other anti-HIV-1 inhibitors against a mixture of R5-HIV-1_{BaL} and X4-HIV-1_{1049pe}.



Drug interaction with other anti-HIV-1 agents against a 50 : 50 mixture of R5-HIV-1_{BaL} and X4-HIV-1_{1049pe}. PBMC with combination of drugs at serial dilutions were cultured with a 50 : 50 mixture of R5-HIV_{BaL} and X4-HIV-1_{1049pe} for 7 days. Drug interactions analyzed with Bliss Independence method are shown were plotted in 3-D graph (A-F). Combination effects were statistically compared with the value with a single agent (G-L). All combination of AK602 + AMD3100 (G), TE14011 (H), T-20 (I), sCD4 (J) or AZT (K) showed synergistic effect. No significance was observed in combination with IDV compared to IDV alone (L). Combination assays of AK602 with other drug were performed 5 times. Assays with a single drug were conducted 10 times. All assays were conducted in duplicate.

Figure 4 Drug interactions between AK602 and other CCR5 inhibitor against R5-HIV-1_{BaL}.



Drug interactions between AK602 and other CCR5 inhibitors against R5-HIV-1_{BaL}. AK602 + TAK 779 showed mild synergistic effect (A). Additivity was observed with AK602 + SCH-C (B).

Table 2 Summary of drug interaction

virus	combination (+AK602)	(Bliss Independence) Combination Effect (%)	Compared to AK602		ED ₅₀	ED ₉₀
			Test drug	(Combination Index) (p value)		
Ba-L	NVP	8.0x3.1	0.002	0.003	0.74	0.43
	IDV	5.2x2.3	0.002	0.003	0.74	0.43
	DV	6.4x1.9	0.002	0.03	0.64	0.50
	T-20	7.2x1.9	0.002	0.002	0.78	0.66
	SCH-C	4.4x2.3	N.S.	N.S.	1.05	0.58
Ba-L-1049pe	TAK779	3.2x2.2	0.03	N.S.	0.83	0.55
	AMD3100	8.0x4.4	0.002	0.005	0.31	0.16
Ba-L-1049pe	TE14011	8.2x4.5	0.002	0.002	0.56	0.23
	AZT	4.2x2.2	0.003	0.005	0.72	0.46
	IDV	5.2x2.8	0.005	N.S.	0.84	0.60
Ba-L-N4-3	T-20	4.8x4.2	0.005	0.04	0.63	0.27
	sCD4	4.3x1.3	0.002	0.002	0.51	0.32

CI<1: antagonistic, CI=1: additive effect, CI>1: synergistic

Conclusions

The present data suggest that AK602 has favorable anti-HIV interactions with other existing anti-HIV drugs and the compound may represent a potential therapeutic for HIV treatment. Most potent synergism with AK602 with two CXCR4 inhibitors appeared to be explained by the report by Singer *et al* (*J Virol*. 75:3779, 2001) that CD4, CCR5 and CXCR4 are present on target cells as adjacently apposed homogenous microclusters.