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Evaluation Of The Antiviral Activity And Cytotoxicity Of Nelfinavir In Combination With Reverse Transcriptase And Protease Inhibitors in an *In Vitro* Acute HIV-1 Infection Model.

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Nelfinavir (formerly known as AG1343) is a potent and selective, non-peptidic inhibitor of HIV-1 protease (K_i = 2 nM) that was developed by protein structure-based design. *In vitro*, nelfinavir was effective at inhibiting the replication of laboratory, clinical and reverse transcriptase inhibitor-resistant strains of HIV with a mean ED_{50} of 22 nM and a mean ED_{95} of 60 nM. As a rationale for potential combination therapy regimens, we evaluated the antiviral efficacy and cytotoxicity of nelfinavir in both 2-drug combinations with zidovudine (AZT), 2'3'-dideoxycytidine (ddC), stavudine (d4T), lamuvidine (3TC), didanosine (ddI), saquinavir, ritonavir, or indinavir and a 3-drug combination with AZT and 3TC against an acute HIV-1 RF infection of CEM-SS cells. Data was analyzed using a universal response surface approach which fits the data to a concentration-effect surface using nonlinear regression as well as by the method of Pritchard and Shipman. These analyses indicate that the combination of nelfinavir with either AZT, ddC, d4T, 3TC, or ddI demonstrated additivity to statistically significant synergism. The interaction of nelfinavir with saquinavir and ritonavir was at least additive while the interaction of nelfinavir with indinavir was slightly antagonistic. Little or no cellular cytotoxicity was seen with any drug alone or in combination. Preclinical evaluation of the interaction of different drugs may contribute to the rational design of appropriate therapeutic regimens. Moreover, these results suggest that administration of combinations of the appropriate doses of nelfinavir with specific compounds *in vivo* may result in enhanced antiviral activity with no additional cytotoxicity at lower, and perhaps less cytotoxic, doses of compounds.

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The combination of Tat and NF-κB inhibitor is more effective to suppress the HIV reactivation than either alone

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HIV regulatory protein Tat is a fascinate target of anti-HIV chemotherapy, though HIV proliferation is not suppressed completely by inhibition of Tat function. Recently it comes clearly that a cellular factor NF-κB plays important role in HIV reactivation. In this paper, we did a combination trial with a Tat inhibitor GCPK[(1)] and a NF-κB inhibitor α-Lipoic acid (LA)[(2)]. We chose LA as NF-κB inhibitor in this experiments, because LA has the most potent activity among our tested NF-κB inhibitors. At acute infection model, this combination showed additive manner. 10μg/ml of LA and 2μg/ml of GCPK inhibited HIV proliferation completely. At the chronic infection model, this combination showed synergistic manner. 0.5mg/ml of LA and 1μg/ml of GCPK suppressed the HIV reactivation from TNF-α stimulated OM-10.1 cells significantly (112pg/ml of p24 amount vs. 12658pg/ml of p24 amount by no treatment). by single use of GCPK (1μg/ml) and LA (0.5mg/ml) , the p24 amount produced was 6265pg/ml and 1975pg/ml respectively. We propose that the combination of NF-κB inhibitor and Tat inhibitor is better way than either single use of 2 compounds for suppressing the HIV proliferation and reactivation *in vitro*.

(1) Kira et al., Antiviral Res. 32, 55-62 (2) Merin et al., FEBS letter, 394, 9-13

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Three-drug combination of MKC-442, 3TC and AZT *in vitro*: Potential approach towards effective chemotherapy against HIV-1

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MKC-442 (6-benzil-1-ethoxymethyl-5-isopropyluracil), a potent non-nucleoside reverse transcriptase inhibitor, is a promising candidate for the treatment of HIV-1 infection and now being under clinical trials. We studied the *in vitro* activity of MKC-442 against HIV-1 replication in a three-drug combination regimen with zidovudine (AZT) and lamivudine (3TC). Drug-drug interactions in MT-4 cells and peripheral blood mononuclear cells (PBMCs) infected with HIV-1 (III_B strain) were evaluated. The multiple drug effect analysis based on the median-effect principle was applied, and the combination indices were calculated with a computer software program. The occurrence of viral breakthrough was investigated during a long-term culture of HIV-1-infected MT-4 cells. When MKC-442 was combined with 3TC and AZT, they synergistically suppressed HIV-1 replication in MT-4 cells over a wide range of doses irrespective of the end points for synergy calculations. Similar results were also obtained in PBMCs. An arbitrary combination ratio of 10:100:1 for MKC-442: 3TC:AZT showed stronger synergism than any other ratios examined. As a result of synergy in the three-drug combination, the dose of single drugs could be reduced by 4- to 24-fold. The three-drug combination markedly delayed or even completely suppressed the HIV-1 replication at least for 40 days. Virus emerged in the presence of the three drugs at lower doses, yet it did not contain any amino acid mutations in the sequenced reverse transcriptase region and retained full sensitivity to all three drugs. Our results demonstrate a potential efficacy of MKC-442 in combination with 3TC and AZT, and the three-drug combination should be considered for the treatment of AIDS patients.

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THE COMBINED PRESENCE OF THE QUINOXALINE HBY097 AND 3TC RESULTS IN POTENT SUPPRESSION OF AZT-RESISTANT HIV-1 STRAINS

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The non-nucleoside reverse transcriptase inhibitor (NNRTI) quinoxaline HBY 097 has been subject of clinical trials in HIV-1-infected individuals. This drug has been shown to inhibit the replication of HIV-1 strains in the nanomolar concentration range. We have now examined the effects of different combinations of HBY 097 and lamivudine (3TC) on two different AZT-resistant HIV strains. Replication of both HIV-1/AZT-2 [containing the 41-Leu and 215-Tyr mutations in their RT] and HIV-1/AZT-4 [containing the 67-Asn, 70-Arg, 215-Phe and 219-Glu mutations in their RT] strains was markedly inhibited by the combination of 3TC and HBY 097. Virus breakthrough was completely suppressed in the combined presence of 3TC and HBY 097 at concentrations as low as 0.05 and 0.0025 μg/ml, respectively. The virus recovered after exposure to lower compound concentrations had acquired the 103-Glu/Arg, 106-Ala, 138-Lys, 184-Ile or 189-Ile mutations that were added to the genetic AZT-resistance background. All these mutant virus strains retained marked sensitivity to HBY 097. This was confirmed by the potent inhibitory effect of HBY 097 against recombinant mutant RT enzymes containing the NNRTI-specific mutations in a genetic AZT-resistance background. Given the exquisite potency of the concomitant combination of 3TC and HBY 097 in suppressing (mutant) virus replication, this drug combination should be further pursued in clinical trials in HIV-1-infected individuals.