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# Synergistic efficacy of combination of Enfuvirtide and Sifuvirtide, the first and next generation HIV fusion inhibitors

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## Abstract

Enfuvirtide and Sifuvirtide, the first and next generation HIV fusion inhibitors, contain different functional domains and have distinct target sites. Here we found that combination of Enfuvirtide and Sifuvirtide resulted in potent synergism in inhibiting HIV-1-mediated cell-cell fusion and infection by X4 and R5 as well as Enfuvirtide-resistant HIV-1 strains. These findings suggest that application of Enfuvirtide and Sifuvirtide in combination may improve their efficacy and resistant profile, leading to reduction of the dosage and frequency of drug use.

In general, a first generation drug would be replaced by a next generation drug in clinical use because the latter may have improved efficacy, pharmaceutical properties or drug-resistant profiles. However, our study here suggests that the first and next generation HIV fusion inhibitors - Enfuvirtide [1] and Sifuvirtide [2] may be preferably used in combination rather than used separately.

Enfuvirtide, which was designed based on the sequence of HIV-1 gp41 C-terminal heptad repeat (CHR) [3,4], was approved by the FDA of the United States in 2003 as the first member of a new class of anti-HIV drugs - HIV fusion inhibitor for treatment of HIV/AIDS patients who are refractory to current antiretroviral drugs. However, its clinical use is limited because it must be injected twice daily and 90 mg per dose, resulting in serious injection site reactions and financial burden for patients, due to its relatively low potency and short half-life. Rapid emergence of Enfuvirtide-resistance in some Enfuvirtide-treated patients is the main cause of treatment failure [5].

Recently, a new generation HIV fusion inhibitor - Sifuvirtide was designed by modification of Enfuvirtide sequence, including change of the sequence of the N-terminal heptad repeat (NHR)-binding domain (NBD), deletion of the lipid-binding domain (LBD) and addition of the pocket-binding domain (PBD) to improve the stability, pharmacokinetics, and antiviral potency [2]. Our previous studies have shown that Sifuvirtide is more potent than Enfuvirtide in inhibiting infection by a wide range of laboratory-adapted and primary HIV-1 strains, including those resistant to Enfuvirtide [2]. Sifuvirtide has longer half-life than Enfuvirtide [6]. We have also demonstrated that Sifuvirtide and Enfuvirtide, although both are HIV fusion inhibitors, have different functional domains and distinct target sites [2]. It is believed that use of combinations of two or three drugs with different target sites or mechanisms of action may

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exhibit synergistic efficacy [7]. We thus proposed that combination of Sifuvirtide and Enfuvirtide might result in synergistic anti-HIV effect.

In the present study, we first compared the inhibitory activity of Sifuvirtide and Enfuvirtide when they were tested alone or in combination on HIV-1-mediated cell-cell fusion using a dye transfer assay as previously described [3]. The concentration of a peptide causing 50% inhibition ($IC_{50}$) and combination index (CI) were calculated using CalcuSyn software (Biosoft) [7]. The results are shown in Table 1. Combination of Enfuvirtide and Sifuvirtide resulted in strong synergism in blocking HIV-1-mediated membrane fusion with CI of 0.182 and potency increase >600%. Subsequently, we determined the potential synergistic effect on inhibition of infection by HIV-1 R5 (Bal) and X4 (IIIB) and Enfuvirtide-resistant strains ($NL4\text{-}3_{V38A}$ and $NL4\text{-}3_{V38A/N42D}$) by measuring p24 production as previously described [2, 8]. When tested separately, both Enfuvirtide and Sifuvirtide have similar antiviral efficacy against HIV-1 X4 and R5 strains, but Enfuvirtide was much less effective than Sifuvirtide to inhibit Enfuvirtide-resistant variants. The combination of Enfuvirtide and Sifuvirtide exhibited synergism in inhibiting infection by HIV-1 Bal and IIIB with potency increase about 240%–300% and CI < 0.6. Strikingly, Enfuvirtide, when combined with Sifuvirtide, became highly effective against Enfuvirtide-resistant HIV-1 strains with reduction of $IC_{50}$ values about 8–15-folds. Similarly, the *in vitro* antiviral efficacy of Sifuvirtide against Enfuvirtide-resistant HIV-1 strains was also significantly increased (Table 1).

Our previous studies have shown that, although both Enfuvirtide and Sifuvirtide are peptidic HIV fusion inhibitors, they inhibit HIV infection by binding to distinct target sites. Sifuvirtide, which contains a PBD and a NBD, can interact with viral gp41 hydrophobic pocket and NHR region to form stable six-helix bundle and block gp41 fusion core formation [2]. However, Enfuvirtide does not contain a PBD but has a LBD. Therefore, Enfuvirtide cannot interact with gp41 pocket to form stable six-helix bundle. It inhibits HIV-1 gp41-mediated membrane fusion by binding to gp41 NHR via its NBD and lipid membranes through its LBD [2]. Because Enfuvirtide and Sifuvirtide have different target sites, their combination thus lead to a strong synergistic anti-HIV effect. Based on these results, we would suggest that Enfuvirtide and Sifuvirtide be used in combination in clinics for treatment of the HIV/AIDS patients, especially those who have failed to respond to Enfuvirtide. Application of other drugs of different generations in combination, if they have different target sites or mechanisms of action, may also have synergistic efficacy.

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**Table 1**

Combination index (CI) and potency increase (%) for inhibition of HIV-1-mediated cell-cell fusion and HIV-1 infection by combination of Enfuvirtide and Sifuvirtide

| Inhibition of | CI[#] | Enfuvirtide | | | Sifuvirtide | | |
|---|---|---|---|---|---|---|---|
| | | $IC_{50}$(nM) | | Potency increase | $IC_{50}$ (nM) | | Potency increase |
| | | Alone | In mixture | | Alone | In mixture | |
| *cell-cell fusion* | | | | | | | |
| | 0.182 | 27.29 | 3.83 | 612% | 7.53 | 0.96 | 684% |
| *infection by HIV-1* | | | | | | | |
| Bal (R5) | 0.449 | 3.18 | 0.66 | 382% | 2.70 | 0.66 | 309% |
| IIIB (X4) | 0.547 | 15.63 | 4.65 | 236% | 7.90 | 2.32 | 241% |
| $NL4\text{-}3_{V38A}$ [*] | 0.36 | 313.04 | 36.19 | 765% | 1.58 | 0.45 | 249% |
| $NL4\text{-}3_{V38A/N42D}$ [*] | 0.123 | 2,645.98 | 170.25 | 1,454% | 40.50 | 2.13 | 1,801% |

[*] Enfuvirtide-resistant HIV-1 strain. Data are representative of two separate experiments. Each sample was tested in triplicate and the mean values were presented.

[#] CI <1: synergism (<0.1: very strong synergism; 0.1–0.3: strong synergism; 0.3–0.7: synergism; 0.7–0.85: moderate synergism; and 0.85–0.90: slight synergism); and CI > 1: antagonism [7].

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