Synergistic In Vitro Antiretroviral Activity of a Humanized Monoclonal Anti-CD4 Antibody (TNX-355) and Enfuvirtide (T-20)

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Recently, antiretroviral agents directed at several steps involved in viral entry have been shown to reduce viral replication in vitro and in vivo. We have demonstrated a high level of in vitro synergistic antiretroviral activity for two entry inhibitors that are directed at sequential steps in the entry process.

Combination chemotherapy has had a profound effect on human immunodeficiency virus type 1 (HIV-1)-associated morbidity and mortality (8). Although there are now more than 20 approved antiretroviral chemotherapeutic agents, there is significant overlap in resistance patterns among these agents, effectively limiting the number of sequential antiretroviral regimens available to a given patient. Recent clinical trials have demonstrated that agents that either block the binding of HIV-1 to its chemokine coreceptors or prevent reconfiguration of the gp41 molecule significantly reduce the replication of HIV-1 in vivo (5, 6, 13, 14). These in vivo observations have been complemented by a series of in vitro observations that demonstrate that antiviral agents acting at several steps of the entry cascade exhibit substantial antiviral synergism (15, 16). TNX-355 (formerly hu5A8) is a humanized monoclonal antibody that binds to a unique epitope in domain 2 of the CD4 molecule that is involved in the conformational change required for entry into target cells following binding of the virus to the CD4 molecule (2, 7). We report here in vitro studies that demonstrate the synergistic activity of TNX-355 and enfuvirtide against HIV-1.

Peripheral blood mononuclear cells were isolated from HIV-1-uninfected donors by Ficoll-Hypaque density gradient centrifugation and grown in RPMI 1640 medium supplemented with 20% fetal calf serum, 5% interleukin-2, and 5 μg/ml phytohemagglutinin (R-3 medium). Three-day phytohemagglutinin blasts (2 × 10⁶) were incubated with HIV-1 (100% tissue culture infective doses) and TNX-355, enfuvirtide, or both agents in 2.0 ml of medium overnight in 24-well tissue culture plates at 37°C in a humidified 5% CO₂ atmosphere. Peripheral blood mononuclear cells were washed three times in phosphate-buffered saline and resuspended in 2 ml of R-3 medium with replacement of the antiviral compound(s) being tested. HIV-1 p24 antigen production was assessed on days 4 and 7 of culture in cell-free supernatant fluid from each well by enzyme-linked immunosorbent assay (Beckman Coulter, Miami, FL). Fresh R-3 medium with appropriate antiviral compound concentrations replaced the 0.5 ml of supernatant removed for p24 assay on day 4. Primary HIV-1 isolates (302076, 302077, 302143, 302054, and 301714) were provided by the National Institutes of Health AIDS Reference Reagent Repository. Human T-cell lymphotropic virus strain IIIB (HTLV-IIIB) was provided by Robert Gallo (Institute of Human Virology, Baltimore, MD). TNX-355 (Tanox, Inc., Houston, TX) was used at concentrations of 2.0, 0.4, 0.08, 0.016, 0.0032, and 0.00064 μg/ml. Enfuvirtide (T-20; Trimeris, Inc., Durham, NC) was used at concentrations of 1.0, 0.2, 0.04, 0.008, 0.0016, and 0.00032 μg/ml. The 50% neutralization concentration (IC₅₀) was calculated by using the Chou dose-effect equation (3). In synergy studies, virus was cultured in the presence of either a single drug or a combination of drugs in a checkerboard combination design of concentrations over the range outlined above. IC₅₀s and combination index (CI) values were calculated by using the Chou dose-effect equation (3). By convention, a CI of <0.9 indicates synergy, 0.9 < CI < 1.1 indicates additive activity, and a CI of >1.1 indicates antagonism.

The antiviral activities of TNX-355 against a variety of laboratory-derived and clinical strains of HIV-1 ranged from 0.13 to 2.0 μg/ml when the antibody was present for only the initial 18 h of the culture period (Table 1). When the antibody was replenished throughout the culture period, the mean IC₅₀s were significantly lower. Synergistic antiretroviral activity between TNX-355 and enfuvirtide was demonstrated in each experiment. The results of a representative experiment are

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IC₅₀ (μg/ml)</th>
<th>Single exposure</th>
<th>Continuous exposure</th>
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<tbody>
<tr>
<td>HTLV-IIIB</td>
<td>1.30</td>
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<tr>
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<tr>
<td>HIV-1 302054</td>
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<tr>
<td>HIV-1 301714</td>
<td>1.82</td>
<td>0.30</td>
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<tr>
<td>HIV-1 NL4-3</td>
<td>0.73</td>
<td>0.067</td>
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Mean ± SD: 1.02 ± 0.70, 0.09 ± 0.10

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activity was defined as a CI of 0.12 compared to a CI of 0.9. Antagonism was defined as a CI of >0.9. CIs: T-20 IC50, 0.023; T-20/5A8, 0.012. Data shown are from a representative experiment using the HTLV-IIIB strain of virus harvested after 4 days of incubation. Individual data points represent percent inhibition compared to untreated controls using means from three wells per drug combination. CIs were calculated by the Chou median-effect principle (3).

presented in Table 2. Table 3 presents CIs calculated for each of the viral strains tested. In either scenario, the mean CI was significantly less than 0.9. CIs of 0.13 to 0.44 were observed when the two agents were included during the initial 18 h of tissue culture in checkerboard titrations against the viral strains tested. CIs indicated a greater degree of synergy when both agents were present throughout the culture period.

In a recently completed proof-of-concept study, a single dose of TNX-355 resulted in a mean plasma HIV-1 RNA decline of 1.25 log10 in a group of multidrug-experienced patients, including those for whom highly active antiretroviral therapy had failed (4). The 5A8 antibody does not result in the clearance of CD4 cells in vivo and does not measurably interfere with CD4 cell function (1, 9–12). This demonstration of the in vivo antiretroviral activity of TNX-355 raises the possibility that a novel step in the entry process may be exploited in the development of antiretroviral chemotherapeutic approaches that might be of particular utility in patients with viral strains that have developed resistance to antiviral drugs directed at the viral reverse transcriptase and/or serine protease. In the studies reported here, we have demonstrated that TNX-355 exhibits synergistic antiretroviral activity with enfuvirtide against both laboratory and clinical strains of HIV-1. That this monoclonal antibody acts synergistically with an agent acting at a distal step in the entry cascade supports the antiviral strategy of combining agents directed at sequential steps of the viral entry process. As chemokine receptor antagonists enter clinical development, three-way antiretroviral synergy efforts directed at the viral entry process should also be examined.

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REFERENCES


