Synergistic Combinations of the CCR5 Inhibitor VCH-286 with Other Classes of HIV-1 Inhibitors

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Here, we evaluated the in vitro anti-HIV-1 activity of the experimental CCR5 inhibitor VCH-286 as a single agent or in combination with various classes of HIV-1 inhibitors. Although VCH-286 used alone had highly inhibitory activity, paired combinations with different drug classes led to synergistic or additive interactions. However, combinations with other CCR5 inhibitors led to effects ranging from synergy to antagonism. We suggest that caution should be exercised when combining CCR5 inhibitors in vivo.

As more members of this class of entry inhibitors make their way through the process of development for use in HIV treatment, it is important to evaluate their interactions and rule out any antagonistic effects (4). Therefore, in this work, we aimed to evaluate the in vitro interactions of a new candidate CCR5 inhibitor, VCH-286, with other members of the same class, MVC and VVC, and also with representative candidates from other classes of HIV inhibitors.

We first established the inhibitory effects of the three CCR5 inhibitors MVC, VVC, and VCH-286 using a dose-response inhibitory assay against two HIV-1 R5 isolates, the laboratory strain HIV-1BAL and the clinical isolate HIV-1CC1/85 (18–21). Viral infections were carried out on total peripheral blood mononuclear cells (PBMCs) from three HIV- and hepatitis B virus-seronegative donors (all participants were adults and signed written informed consent approved by the Centre de Recherche du Centre Hospitalier de l’Université de Montréal [CRCHUM] institutional review boards). The cells were isolated by Ficoll-Paque gradient separation and stimulated for 3 days with phytohemagglutinin (PHA) (1 mg/ml) and interleukin-2 (1 μg/ml) in 24-well tissue culture plates, followed by infection with 3,000× the tissue culture infectious doses (TCID) of the HIV-1 R5 viruses. As shown in Fig. 1B and C, viral replication of both HIV strains was readily inhibited by the three CCR5 inhibitors when monitored by the production of the viral core protein p24 (measured by enzyme-linked immunosorbent assay [ELISA]). The 50% inhibitory concentrations (IC50s) (calculated by dose-effect analysis using the CalcuSyn software [Biosoft, Cambridge, United Kingdom]) were used to determine the antiviral activities of the three drugs, as these compounds act at the cell surface and are not dependent on cellular uptake and metabolism. The IC50s against the HIV-1R5 strain for MVC, VVC, and VCH-286 were 1.85 nM, 3.38 nM, and 0.23 nM, respectively.

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The IC₅₀s against HIV-1 CC1/85 for MVC, VVC, and VCH-286 were 4.39 nM, 3.78 nM, and 0.34 nM, respectively (Table 1). Of note, no toxicity was observed in the uninfected PBMCs with concentrations up to 1,000 nM with any of these three drugs. These results are thus consistent with earlier reports of strong antiviral activities of MVC and VVC against both HIV-1BAL and HIV-1CC1/85 infections (12, 22). Moreover, VCH-286 showed a significant inhibition of viral replication at drug concentrations that were lower than those of the two other drugs (i.e., IC₅₀s 8- to 14-fold lower than those of MVC and VVC).

FIG 1 (A) Chemical structure of the new CCR5 inhibitor VCH-286, a citrate salt. (B) Inhibitory effects of VCH-286 (left), MVC (middle), and VVC (right) on HIV-1BAL. (C) Inhibitory effects of VCH-286 (left), MVC (middle), and VVC (right) on HIV-1CC1/85. The core viral protein p24 was measured from the culture supernatant by commercial enzyme-linked immunoassay (PerkinElmer) at day 7 postinfection (mean ± SD from three independent experiments). *, P < 0.05 calculated by paired t test (comparing the p24 production by HIV-infected cells with each drug concentration relative to infected nontreated cells). Cell viability was assessed by the exclusion method using the Trypan blue dye.
We further evaluated the impact of drug interactions through paired combinations between MVC, VVC, and VCH-286 on HIV replication, using the same experimental settings. Drug combinations using CCR5 inhibitors may represent an interesting approach, as the association and dissociation rates to the CCR5 receptor may differ between the drug candidates, thus providing a pharmacodynamic advantage in maintaining adequate receptor occupancy (23). Therefore, we opted to define whether different drug combinations would result in synergistic effects. Synergism takes place when the combination is more effective than single-agent use; one of the agents increases the actions of the second drug. Antagonism is when the combination is less effective than with the use of single agents; one of the agents counteracts the actions of the other. We also employed a multiple-drug effect analysis. This multiple-drug effect analysis is based on the median-effect principle and the isobologram technique (24). While the combination indices (CI) CI_{50}, CI_{75}, and CI_{90} of any given combination of two drugs provide information on the nature and extent of drug interaction at the IC_{50}, IC_{75}, and IC_{90} of each drug, respectively. A combination was defined as synergistic when the CI value was <1, additive when the CI was 1, and antagonistic when the CI was >1, as described earlier (25). Combinations of CCR5 inhibitors showed interactions ranging from synergy to antagonism, as illustrated by the combination indices (CI) shown in Table 2. The interaction of MVC and VCH-286 was highly synergistic under all tested concentrations against both viral isolates, with CI_{90} values of 0.41 (mean, 0.47 and 0.35 from two independent experiments) and 0.43 (mean, 0.44 and 0.42) for HIV-1_{BAL} and HIV-1_{CC1/85}, respectively. In contrast, combinations of MVC with VCH showed highly antagonistic interactions against both HIV isolates under the different inhibitory concentrations tested, with CI_{90} values of 5.61 (mean, 5.99 and 5.24 from two independent experiments) and 1.86 (mean, 1.37 and 2.34) for HIV-1_{BAL} and HIV-1_{CC1/85}, respectively. Meanwhile, the interaction between VVC and VCH-286 was additive, with a CI_{90} value of 1.08 (mean, 1.1 and 1.05 from two independent experiments) against HIV-1_{BAL}. However, this same combination performed in an antagonistic fashion against HIV-1_{CC1/85} with a CI_{90} value of 2.22 (mean, 2.52 and 1.92 from two independent experiments).

VCH-286 was further evaluated in dual combinations with representative drugs from each of the currently approved antiretroviral classes: the nucleoside reverse transcriptase inhibitors zidovudine (AZT) and lamivudine (3TC), the nonnucleoside reverse transcriptase inhibitors nevirapine (NVP) and efavirenz (EFV), the protease inhibitors lopinavir (LPV) and saquinavir (SQV), the integrase inhibitor raltegravir (RTG), and the fusion inhibitor enfuvirtide (Fuzeon, T-20) (Table 3). The laboratory-adapted strain HIV-1_{BAL} and the clinical isolate HIV-1_{CC1/85} were both susceptible to almost all the antiretroviral drug combinations with VCH-286 used in this study, and synergistic or additive interactions were observed, as shown in Table 3. The synergistic and additive effects of the combination of VCH-286 with other drug candidates are consistent with our earlier observations (26) and those of others (12) on the combination of the CCR5 inhibitors in vitro. Only two exceptions with moderate and significant antagonistic effects were observed for HIV-1_{BAL} and HIV-1_{CC1/85} when combining VCH-286 with lopinavir and 3TC, respectively (Table 3). The CI_{90} for the combination of VCH-286 with lopinavir against HIV-1_{BAL} was 1.39 (mean, 1.69 and 1.09 from two independent experiments), whereas the CI_{90} for the combination of VCH-286 with 3TC against HIV-1_{CC1/85} was 2.03 (mean, 2.1.2 and 1.85). Although we did not study the mechanism(s) underlying the clear antagonism between the CCR5 inhibitor VCH-286 and the nucleoside reverse transcriptase inhibitor (NRTI) 3TC, this might be related to a potential interference with the cell activation process. The NRTI 3TC is known to be dependent on the cellular machinery in order to be transformed from the initial monophosphate to the triphosphate active form (27), a step that might be affected by the interference with CCR5 signaling by VCH-286. On the other hand, the moderate antagonism with lopinavir might be related to an unappreciated low level of cytotoxicity mediated by the drug combination. Of note, the cytotoxicities for all single drugs and drug combinations were assessed by treating noninfected cells with the highest concentrations used in the current study. Cell viability was tested by the Trypan blue exclusion method and showed negligible effects.

Altogether, our results clearly show that the new CCR5 inhibitor VCH-286 performed well when used as a single agent, with IC_{50}s 8- to 14-fold lower than those of the two other CCR5 inhibitor drugs. It also showed favorable combination indexes with

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**TABLE 1** IC_{50}s obtained for MVC, VVC, and VCH-286 against the R5 viruses HIV-1_{BAL} and HIV-1_{CC1/85}.<sup>a</sup>

<table>
<thead>
<tr>
<th>Drug</th>
<th>HIV-1_{BAL} IC_{50} (nM)</th>
<th>HIV-1_{CC1/85} IC_{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC</td>
<td>1.85</td>
<td>4.39</td>
</tr>
<tr>
<td>VVC</td>
<td>3.38</td>
<td>3.78</td>
</tr>
<tr>
<td>VCH-286</td>
<td>0.23</td>
<td>0.34</td>
</tr>
</tbody>
</table>

<sup>a</sup> IC_{50}, 50% inhibitory concentration; MVC, maraviroc; VVC, vicriviroc.

**TABLE 2** Combination indices for MVC, VVC, and VCH-286 against the R5 viruses HIV-1_{BAL} and HIV-1_{CC1/85}.<sup>a</sup><sup>b</sup>

<table>
<thead>
<tr>
<th>Virus</th>
<th>Drug combination</th>
<th>C_{I_{50}} SD</th>
<th>C_{I_{75}} SD</th>
<th>C_{I_{90}} SD</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1_{BAL}</td>
<td>MVC + VCH-286</td>
<td>0.76 0.05</td>
<td>0.56 0.03</td>
<td>0.41 0.08</td>
<td>Synergy</td>
</tr>
<tr>
<td></td>
<td>MVC + VVC</td>
<td>9.53 0.629</td>
<td>7.49 0.377</td>
<td>5.61 0.53</td>
<td>Antagonism</td>
</tr>
<tr>
<td></td>
<td>VVC + VCH-286</td>
<td>0.96 0.06</td>
<td>1.055 0.007</td>
<td>1.08 0.03</td>
<td>Additive</td>
</tr>
<tr>
<td>HIV-1_{CC1/85}</td>
<td>MVC + VCH-286</td>
<td>0.61 0.01</td>
<td>0.50 0.002</td>
<td>0.43 0.014</td>
<td>Synergy</td>
</tr>
<tr>
<td></td>
<td>MVC + VVC</td>
<td>10.17 0.7</td>
<td>4.22 0.54</td>
<td>1.86 0.68</td>
<td>Antagonism</td>
</tr>
<tr>
<td></td>
<td>VVC + VCH-286</td>
<td>0.68 0.23</td>
<td>1.91 0.098</td>
<td>2.22 0.42</td>
<td>Antagonism</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are presented as the means from two independent experiments (three replicates per condition for each experiment) ± standard deviations (SD). The ranges of doses used for MVC, VVC, and VCH-286 were as follows: 0.0128, 0.064, 0.32, 1.6, 8, 40, 200, and 1,000 nM.

<sup>b</sup> Combination index (CI) interpretation: <1, synergy; 1, additive; and >1, antagonism.
Similarly, Murga et al. (32) observed a significant synergy for the combination with three small-molecule CCR5 inhibitors (maraviroc, TAK-779), with CI values from 0.36 to 0.61, but weaker interactions were observed with the combination of MVC and other members from the same class, such as TAK-779 and SCH-C, leads to mild synergism and additivity, respectively. Our results are therefore consistent with earlier reports on the combination of CCR5 inhibitors. Nakata et al. (31) reported that a combination of the CCR5 inhibitor aplaviroc and other members from the same class, such as TAK-779 and SCH-C, leads to mild synergism and additivity, respectively. Similarly, Murga et al. (32) observed a significant synergy for the humanized CCR5 monoclonal antibody (MAb) PRO 140 in combination with three small-molecule CCR5 inhibitors (maraviroc, vicriviroc, and TAK-779), with CI values from 0.36 to 0.61, but additive effects were observed with the combination of MVC and VVC.

In vitro studies of drug interactions have proven to be beneficial in predicting which drug combination regimens should be tested in a clinical setting (12–14). In the present study, we also evaluated the interactions between VCH-286 and representatives from each class of currently available antiretroviral agents in vitro. We have found that in the nanomolar range, VCH-286 exerted antagonistic effects against HIV-1 R5 viruses when it was combined with AZT, NVP, SQV, RTG, and T-20.

In conclusion, our current study highlights the efficacy of VCH-286 as a new antiviral agent inhibiting HIV-1 binding to CCR5. It has favorable drug interactions with antiretroviral drugs (ARVs) used in the clinic to treat HIV/AIDS, such as reverse transcriptase, protease, integrase, and fusion inhibitors, thus suggesting that VCH-286 may be a useful anti-HIV drug in combination therapy. However, we raise the possibility that antagonistic effects with the combination of CCR5 inhibitors, including this new drug candidate, may take place in vivo; hence, caution should be exercised when considering this type of combination in a potential treatment regimen.

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