50

Mechanism of inhibition by piperazinyloxoquinoline derivatives as novel HIV-1 transcription inhibitors

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Piperazinyloxoquinoline derivatives have proved inhibitory to HIV-1 replication in both acutely and chronically infected cells through their selective inhibition of HIV-1 gene expression. To get further insight into the mechanism of action, we examined whether K-12, the most potent congener of the series, could inhibit cellular transcription factors that affect the expression of HIV-1 transcription. In gel mobility-shift assays, K-12 was not inhibitory to tumor necrosis factor (TNF)-α-induced translocation of nuclear factor κB (NF-κB), a potent activator of HIV-1 gene expression, in OM-10.1 cells at concentrations up to 4 μM. At this concentration, the replication of HIV-1 was completely suppressed in the host cells. Furthermore, the intracellular level of another transcriptional factor Sp1 was not reduced by the treatment with K-12. The binding of neither NF-κB nor Sp1 to its target DNA was directly affected by the presence of compound. A cotransfection experiment with a Tat-expressing plasmid and a HIV-1 long terminal repeat (LTR)-driven chloramphenicol acetyltransferase (CAT)-expressing plasmid revealed that K-12 did not exert its anti-HIV-1 activity through the inhibition of HIV-1 Tat function. This conclusion was further confirmed by the fact that the compound was also inhibitory to the replication of murine retrovirus (LP-BM5), which has no accessory genes such as *tat* and *rev*. These results suggest that piperazinyloxoquinoline derivatives interact with an cellular factor (or factors) necessary for transcription of viral DNA, yet NF-κB and Sp1 are unlikely to be primary targets for inhibition of HIV-1.

51

HUMAN IMMUNODEFICIENCY VIRUS gp120 AS THE PRIMARY TARGET OF ACTION OF AR177 (ZINTEVIR)

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AR177 (Zintevir) is a potent inhibitor of the replication of multiple laboratory strains of HIV-1 in human T-cell lines, peripheral blood lymphocytes and macrophages. The mode of action of AR177 has been attributed, at least in part, to its anti-integrase activity, since it is able to inhibit the integration reaction at a 50% inhibitory concentration of 0.4 μg/ml, roughly the same as the 50% effective concentration (EC_{50}) for its anti-HIV activity. We have found, however, that AR177 inhibits syncytium formation between MOLT 4 cells and persistently infected HUT cells at an EC_{50} of 2.5 μg/ml, roughly 5-fold than that required to inhibit HIV replication. Furthermore, cytometric analysis showed that AR177 at 25 μg/ml interferes with the binding of the monoclonal antibody 9284 (directed to the V3 loop of gp120) to $HIV(III_B)$-infected HUT-78 cells. To characterize the site/target of intervention by AR177, we have selected HIV-1 (NL4-3) strains resistant to AR177. The resistant phenotype was associated with the emergence of mutations in the gp120 molecule of the resistant virus. DNA sequence analysis revealed the mutations K148E, Q278H K290Q, F391I and a deletion of 5 amino acids at positions 364 to 368 (FNSTW) in the V4 region of gp120 of the resistant strain but were not present in the wild-type HIV strain. Here we also show that selection of drug-resistant strains while taking a relative long time to develop may also select for strains with lesser replicative capacity. No mutations were found in the integrase gene. AR177 has proven active against various laboratory strains and clinical isolates of HIV, when used either alone or in combination with other HIV inhibitors. Our data suggest that the primary target of anti-HIV action of AR177 is the viral envelope glycoprotein gp120.

52

Inhibition of RRE-Rev interaction by RNA-binding polycations. Ke Li, David W. Boykin, Alvind Kumar, C. Ted Rigl and W. David Wilson
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Increasing evidence has shown that specific interactions between viral proteins and RNA genome, such as the stem-bulge-loop structures RRE and TAR of HIV-1, are essential for viral gene replication and are thus attractive targets for antiviral drug design. In our attempt to design structure-specific RNA-binding antiviral agents, several series of polycationic ligands were synthesized. Their RNA vs. DNA binding was evaluated by thermal denaturation (ΔTm) with RNA [poly(A).poly(U)] and DNA [poly(dA)·poly(dT)] polymers, and the inhibitory activity on RRE-Rev interaction was analyzed by gel shift assays. This poster presents the structure-activity relationship studies on 4,4'-bispiperidine polycations which highlights structure features important for preferential RNA binding vs. DNA binding. Our results have shown that compound L3, an amidine derivative of 4,4'-bispiperidine, inhibits Rev-RRE interaction, and its strong RNA binding under the assay condition is important for this inhibition. Modeling studies suggest that these polycations bind in the major groove of RNA.

53

The HIV-1 *nef* transframe protein has significant sequence and structural similarity to chemokines, as assessed by threading (inverse folding), sequence analysis, and molecular modeling.

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As detailed in the accompanying abstract (Taylor et al.), the existence of a novel transframe protein expressed by a -1 frameshift from the HIV-1 *nef* coding region is strongly supported by both *in vitro* and theoretical data. The high degree of conservation of the frameshift heptamer and several UGA codons in the -1 frame cannot be explained by requirements of the *nef* coding sequence alone. Computer analysis suggests that this hypothetical HIV-1 transframe *nef-fs* gene may encode a γ-chemokine homologue. The *nef-fs* sequence conforms to a multiple alignment of ~20 CXC, CC and γ- or C-type chemokines, with 2 conserved UGA codons aligning with the 2 absolutely conserved Cys residues of chemokines. The alignment score for *nef-fs* vs. the entire alignment was > 4.5 SD higher than the average score of randomized sequences of identical size and composition. This similarity was strongly confirmed by threading, using the MatchMaker program of Skolnick et al., which can detect structural similarity even when pairwise sequence similarity is low. Using *nef-fs* as a probe against the protein structure fingerprint database, the top hit was the CXC chemokine interleukin 8 (IL8), at 4.8 SD significance, using a gap penalty of 2 and gap extension penalty of 0.2. This result validated our homology model of *nef-fs* based on the IL8 crystal structure, which was also energetically favorable. Because other viruses (e.g. CMV) are known to encode chemokines, and HIV-1 has recently been shown to use both CC-CKR5 and fusin as coreceptors, the possibility of an HIV-1 encoded chemokine homologue merits further investigation.