Cyclotriazadisulfonamides: promising new CD4-targeted anti-HIV drugs

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It is imperative to continue efforts to identify novel effective therapies that can assist in containing the spread of HIV. Recently acquired knowledge about the HIV entry process points to new strategies to block viral entry. For most HIV strains, the successful infection of their target cells is mainly dependent on the presence of the CD4 surface molecule, which serves as the primary virus receptor. The attachment of the viral envelope to this cellular CD4 receptor can be considered as an ideal target with multiple windows of opportunity for therapeutic intervention. Therefore, drugs that interfere with the CD4 receptor, and thus inhibit viral entry, may be promising agents for the treatment of AIDS. The CD4-targeted HIV entry inhibitors cyclotriazadisulfonamides represent a novel class of small molecule antiviral agents with a unique mode of action. The lead compound, CADA, specifically interacts with the cellular CD4 receptor and is active against a wide variety of HIV strains at submicromolar levels when evaluated in different cell-types such as T cells, monocytes and dendritic cells. Moreover, a strict correlation has been demonstrated between anti-HIV activity and CD4 interaction of about 20 different CADA analogues. In addition, CADA acted synergistically in combination with all other FDA-approved anti-HIV drugs as well as with compounds that target the main HIV co-receptors. In this article, the characteristics of cyclotriazadisulfonamide compounds are presented and the possible application of CADA as a microbicide is also discussed.

Keywords: HIV entry, CADA, CD4 receptor, down-modulation, microbicides

Introduction

One of the effective approaches to prevent and inhibit viral infections is to block host cell receptors that are used by viruses to gain cell entry. Major advances have been made over the past decade in our understanding of the molecular mechanism of human immunodeficiency virus (HIV) entry into cells. The external HIV envelope glycoprotein, gp120, sequentially interacts with two cellular receptor molecules, the CD4 glycoprotein and a chemokine receptor, such as CCR5 or CXCR4, leading to the insertion of the envelope fusogenic domain of gp41 into the host cell membrane which finally results in the fusion between the viral and cellular membranes. Each of these discrete steps in the viral entry process represents a potential target for new antiviral agents.1 When used in HIV prophylaxis, drugs that interfere with the earliest events in the replication cycle may have an advantage over existing therapeutic approaches that target the viral enzymes reverse transcriptase (RT) and protease, as they may prevent virus entry into new target cells and subsequently reduce the number of latent reservoirs for HIV. Current efforts to develop safe and effective HIV entry inhibitors are focused on natural ligands and/or monoclonal antibodies that interfere with gp120/CD4 or gp120/co-receptor interaction. Also, small synthetic compounds obtained either by high-throughput screening of large compound libraries or by structure-guided rational design have recently entered the antiretroviral arena.

CADA compounds

A novel class of small molecule antiviral agents is represented by cyclotriazadisulfonamide (CADA) compounds. The lead compound, CADA (Figure 1a), is a synthetic macrocycle with consistent activity against laboratory adapted and primary clinical isolates of HIV-1, irrespective of chemokine receptor preference.2,3 CADA was shown to be equally active against (i) all drug-resistant viruses (i.e. viruses resistant to RT inhibitors, protease inhibitors and enfuvirtide), (ii) all different HIV-1 subtypes (A, B, C, D, A/E, F, H, O), and (iii) various HIV-2 and SIV strains examined.2,3 We discovered that the anti-HIV mechanism of CADA involved specific interaction with the cellular CD4 receptor, leading to a specific decrease in cell surface and intracellular CD4, a mechanism which is quite unique and so far only described for this class of chemical compounds. CADA also showed activity against human herpesvirus type 7 (HHV-7), a virus for which CD4 has been identified as its main receptor.2 The compound has consistent CD4 down-regulating activity in T-lymphocytes and T cell lines (i.e. MT-4, SupT1, CEM, MOLT-4 and Jurkat), in monocytes,
immature dendritic cells and monocytic cell lines (i.e. THP-1 and U937), and in CD4-transfected cells such as U87 and A2.01. Administration of the drug to CD4-positive cells results in a quantitative down-modulation of the CD4 receptor (i.e. almost 90% reduction in CD4), bringing the CD4 receptor density below the level that is required for efficient HIV infection. Importantly, the anti-HIV activity of CADA correlated with its ability to down-modulate the CD4 receptor expression, a correlation which was also observed with 16 CADA analogues (Figure 1b), further pointing to CD4 receptor down-modulation as the primary and unique mode of anti-HIV action for this group of compounds. CADA did not alter the expression of 15 other cellular receptors examined, nor HIV co-receptors CCR5 and CXCR4. An interesting feature of the cyclotriadazadisulfonamides is the reversible nature of their CD4 down-regulating activity: that is, CD4 expression on the cells is rapidly restored to normal levels after removal of the drug. Time course experiments revealed that CADA differs in its mechanism of action from that of aurintricarboxylic acid, which inhibits HIV-1 infection by a direct interaction with CD4, and PMA, which activates protein kinase C. CADA is assumed to down-regulate CD4 expression at the (post)translational level, although its mechanism of action has not been completely elucidated. Further, pertaining to the promising character of CADA as an anti-HIV agent was the observation that it proved synergistic in its anti-HIV activity when combined with RT inhibitors NRTIs (i.e. zidovudine, lamivudine, zalcitabine and abacavir), NNRTIs (i.e. nevirapine and delavirdine), protease inhibitors (i.e. lopinavir, saquinavir, indinavir, nelfinavir, amprenavir and ritonavir) as well as the gp41 fusion inhibitor T-20 (enfuvirtide), the CXCR4 antagonist AMD3100, and the mannose-specific plant lectins from Galanthus nivalis (GNA) and Hippeastrum hybrid (HHA). When exposed to escalating concentrations of CADA, an HIV-1 (NL4.3) strain was isolated after 40 subcultivations that showed a decreased sensitivity to the compound. The CADA-resistant virus strain was investigated for its sensitivity-resistance profile and showed a significant reduction (6-fold) in sensitivity to the anti-CD4 monoclonal antibody RPA-T4 compared with the wild-type counterpart. Determination of amino acid changes in the viral envelope revealed several mutations in the envelope glycoprotein gp120. Interestingly, two amino acid changes occurred at the CD4-binding domain of gp120 and are now the subject of further mutagenesis experiments.

Targeting the CD4 receptor density

The functional HIV-1 envelope protein complex is a trimeric structure comprising three gp120 surface glycoproteins, each non-covalently attached to one of three subunits of the gp41 transmembrane glycoprotein, resulting in the formation of spikes protruding from the virus surface. Three CD4 binding events are needed to efficiently activate HIV-1 Env trimers for viral entry, further implying that CD4 receptor density has a crucial role in effective HIV infection. It has been reported that the HIV titre in clones of human cervical carcinoma (HeLa) cells expressing different (low) levels of CD4 and infected with laboratory strains of HIV-1 increased with increasing CD4 expression. Also primary HIV-1 isolates infected HeLa-CD4 cell clones that have distinct quantities of CD4 in direct proportion to cellular CD4 expression. It has been shown that laboratory adaptation of T-tropic (X4) HIV-1 may involve corresponding increases in affinities for CD4 and in abilities to infect cells that have relatively little CD4. This may explain why laboratory-adapted HIV-1 isolates infected the panel of HeLa-CD4 cell clones with equal efficiencies regardless of the levels of CD4, with the exception of the clone with the lowest CD4 expression. Probably, for laboratory-adapted HIV-1 strains, above a low threshold of CD4 expression HIV titres do not significantly depend on CD4 levels. In addition, mutations that specifically reduce CD4 affinities of gp120 in a laboratory-adapted HIV-1 strain could convert viral infectivities from relative CD4 independence into the strong CD4 dependence described for X4 clinical isolates which preferentially infect cells that co-express CXCR4 and substantial amounts of CD4. The CD4 and CXCR4 concentration requirements for efficient infections by macrophage-tropic (R5) HIV-1 are interdependent: cells with a large amount of CD4 required only a trace amount of CCR5 for maximal susceptibility to infection by diverse isolates of R5 HIV-1, whereas in cells with a small number of CD4 molecules a much larger amount of CCR5 was needed for infection. Thus, the requirements for each receptor are increased when the other component is present in a limiting amount. Therefore, drugs with CD4 down-modulating activity, such as the CADA compounds, can be successful in HIV entry inhibition. Although CADA treatment does not result in removal of all the CD4 molecules from the cell surface, it decreases the CD4 receptor density below a threshold level that is required for efficient infection.

CADA: HIV trap with no escape?

Although CD4-independent viruses have been described, these are much more sensitive to neutralizing antibodies, which could also explain the rarity of strict CD4-independent HIV variants. Remarkably, these viruses still show higher infectivity and replicative ability when CD4 is expressed on the surface of the target cells. A CD4-lowering drug, such as CADA, not only has a direct antiviral effect, but it can also force the virus to become less dependent on CD4 by changing crucial amino acids in its envelope protein. Theoretically, this virus could then be eliminated more efficiently by neutralizing antibodies. Therefore, resistance...
development against CADA can be even considered as favourable and will allow better immunological responses and virus clearance from the systemic circulation. Experiments are ongoing to prove this novel concept of anti-HIV therapy.

**CADA as a topical microbicide**

Since HIV is primarily a sexually transmitted disease, the potential ability of a single prophylactic application of an entry inhibitor to block the initiation of infection at mucosal surfaces is an attractive approach to reducing transmission of HIV. Microbicides, compounds that can be applied topically to prevent HIV infection upon exposure to the virus, can play a crucial role for effective HIV prevention strategies. An ideal microbicide should be active against a broad spectrum of wild-type and drug-resistant HIV-1 and HIV-2 variants belonging to different subtypes or clades and using different co-receptors. Many antiretrovirals that have been developed are very often too specific and only active against a certain HIV strain or isolate. As all HIV viruses need the cellular CD4 receptor for efficient infection of their target cells, the small molecular weight compound CADA, with a potent and consistent anti-HIV activity against a broad range of HIV variants would be very attractive for further development as a microbicide. In addition, it has been shown that blockade of the CD4 receptor alone (with anti-CD4 MAb as a proof-of-concept) completely inhibited viral infection in human cervical tissue *ex vivo*. Also, the *in vitro* synergistic interaction of CADA with all clinically approved anti-HIV drugs suggests that a combination of CADA with other antiretrovirals should be feasible in microbical gel preparations. Furthermore, the results of the proof-of-concept study in HIV-1-infected patients with TNX-355 demonstrate the feasibility of inhibiting HIV entry *in vivo* by a CD4-specific monoclonal antibody and indicate that targeting a cellular receptor represents a potential treatment approach to HIV.

Thus, with regard to the problems of antiretroviral drug toxic effects, CD4 down-modulators might have a role in prophylaxis as microbicides, an example of a way in which these inhibitors could potentially circumvent pharmacological but also immunological problems. Although CD4 receptors are involved in immune cell development, antigen presentation and T cell activation, the considerations of CD4 down-regulation on normal immune function might now be less constraining. Despite the many challenges of safety, efficacy and clinical application, the successful improvement in the design and development of antiretrovirals capable of inhibiting HIV binding and subsequent viral entry should be considered as an important step forward to combat AIDS.

**References**