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Aminoglycoside-arginine conjugates: novel multifunctional HIV Tat antagonists

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Human immunodeficiency virus type 1 (HIV-1) Tat protein, a potent viral transactivator, is implicated in HIV-1 pathogenesis, not only by its indispensability for virus replication but also by its capacity to prime quiescent T cells for productive HIV-1 infection and induce apoptosis in uninfected T cells. HIV Tat protein is the main regulator of HIV proviral gene transcription, which expresses its function through binding to the TAR RNA element, found at the 5' end of all the primary HIV transcripts. The process involves the formation of a ternary complex between Tat protein, TAR RNA and the cyclin T1 subunit of P-TEFb. This leads to activation of CDK9 kinase, overphopsphorylation of RNA polymerase II, resulting in ~ thousand-fold increase of the HIV gene transcription. The structural basis of Tat binding to TAR is an interaction of the Tat basic arginine-rich domain with the TAR RNA conservative structure. NMR structures of HIV TAR bound to different ligands, *e.g.* peptides that mimic Tat basic region, arginine or argininеamide, show that the RNA bulge structure provides ligands with access to the major groove of TAR, which induces folding in the bulge and formation of unusual base-triples.

Tat could directly or indirectly affect multiple steps in the virus life cycle to facilitate HIV-1 infection, considering its pleiotropic biological properties, such as regulation of both viral and cellular gene expression and modulation of growth of various cell types, as well as its release from infected cells and its acting on bystander uninfected cells in a paracrine fashion. Tat differentially induces CXCR4 and CCR5 chemokine receptor expression in peripheral blood mononuclear cells (PBMCs) and promotes the infectivity of both M- and T-tropic HIV-1 strains in primary human leukocytes, notably in monocytes/macrophages. Tat also induces positive chemotaxis of lymphocytes and macrophages. Tat interacts with growth factor receptors, especially with vascular endotelial growth factor receptor (VEGFR) and stimulates proliferation of endothelial cells, which is one of the key mechanisms in the development of Kaposi sarcoma in AIDS patients. Thus, development of Tat protein inhibitors/antagonists comprises an important direction in anti-AIDS drug discovery.

A new class of small molecules (mw ~ 1000 Da), aminoglycosides-arginine conjugates (AAC), was designed and prepared in our laboratory as Tat basic domain mimetics with predominant TAR RNA targeting. We have shown that these compounds, a tri-arginine derivative of gentamicin C (R3G), a tetra-arginine derivative of kanamycin A (R4K) and a hexa-arginine derivative of neomycin B (NeoR) bind efficiently and specifically to TAR RNA with K_d values in the nanomolar range, which is close to the affinity of Tat-basic peptide. AAC binding sites on TAR RNA were assigned by several footprinting techniques. AAC interact with TAR RNA in the widened major groove, formed by the bulge structure and the neighbouring base pairs of the upper stem portion of TAR, the binding site of Tat protein and Tat-derived peptides. These data are strongly supported by preliminary NMR structural studies.

The conjugates display low toxicity while being transported and accumulated in cell nuclei. AAC inhibit equine infectious anemia virus (EIAV) in infected equine dermal fibroblasts with an EC_{50} (50% effective concentration) of 12.5-20 μM for R3G and 50 μM for R4K, without detectable toxicity up to 1 mM. AAC inhibit HIV-1 proliferation in the infected MT2 cells with an EC_{50} of 6-10 μM for R3G and 3-4 μM for NeoR, and a CC_{50} (50% cytotoxic concentration) of 0.5 and 0.25 mM, respectively. Preliminary studies on the anti-HIV activity of AAC suggest that their main function is inhibition of TAR-dependent trans-activation by Tat protein, but this is not their only activity. AAC inhibited the binding of a monoclonal antibody (12G5) directed to CXCR4 as well

as the intracellular Ca^{2+} signal induced by the chemokine SDF-1a on $CXCR4^{+}$ cells, suggesting that AAC interact with CXCR4, the coreceptor used by T-tropic strains of HIV-1. AAC were shown to inhibit the binding of HIV-1 to MT-4 cells, *i.e.* affecting the early stages of HIV-1 infection by blockade of viral entry to the cells. Recently we have shown that AAC down-regulate the expression of CCR5 and CXCR4 chemokine receptors on PHA-stimulated peripheral blood monocytes.

Being Tat basic region mimetics/antagonists, these conjugates comprise a promising strategy for anti-HIV drug design due to their features: a) their ability to target regulatory pathways of HIV life cycle, b) high affinity and specificity for TAR RNA binding, c) inhibition of T-tropic HIV-1 early infection stages *via* blocking CXCR4 chemokine receptor, and d) low cytotoxicity. Further investigation is aimed to improve specificity and selectivity of the next series of aminoglycoside-arginine conjugates and to develop lead compounds towards specific, highly selective and non-toxic anti-HIV drugs.

Currently we are investigating: a) More efficient aminoglycoside-arginine conjugates, using different aminoglycoside cores along with selective modification of aminoglycosides to achieve compounds with higher specificity and activity. b) The mechanisms of antiviral activity of AAC, in particular, trans-activation inhibition and chemokine receptor-mediated activity. c) The effect of AAC on chemokine receptor expression and early stages of infection. d) The conjugates' activity against HIV-1 laboratory strains and primary clinical isolates from different clades, as well as M-Tropic, T-Tropic and drug resistant HIV-1 strains, using cell lines as well as PBMC. e) Investigating possible selection of resistant HIV-1 mutants, caused by administration of these compounds. f) The affinity of AAC to mutated TAR RNA sequences. g) The antiviral activity of the novel compounds in combination with other anti-HIV compounds, and their capacity to delay the appearance of the HIV resistant virus when used together with other antiviral drugs. h) Inhibition by AAC NF-kappaB, ERK/MAPK, and JNK activation by HIV-1 Tat protein and by HIV-1 infection. i) The efficacy of AAC to inhibit Tat-induced Kaposi sarcoma and endothelial cell proliferation.

Our findings suggest that AAC are antagonists of HIV Tat protein, which is crucial for the productive infection of the virus. Currently there are no antiviral drugs of that function used in the clinic. AAC, being multifunctional Tat antagonists, may act at various targets, and therefore may lower the emergence of HIV resistance to these compounds.

References:

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Patents

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