

35

Anti-HIV-1 Activity of A3G is Enhanced by HSP70

Ryuichi Sugiyama^{1,*}, Yuichiro Habu^{2,3}, Haruki Naganuma¹, Hiroshi Koseki¹, Ayako Furukawa⁴, Takashi Nagata⁴, Masato Katahira⁴, Hiroshi Takaku^{1,3}

¹ Department of Life and Environmental Science, Chiba Institute of Technology, Chiba, Japan; ² Department Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, USA; ³ High Technology Research Center, Chiba Institute of Technology, Chiba, Japan; ⁴ International Graduate School of Arts and Sciences, Yokohama City University, Kanagawa, Japan

By an elucidation the action mechanism of a host factor to HIV-1, it is thought that it is applicable to the medical treatment of HIV-1. Our study focused on A3G which controls a virus duplicate to negative. A3G has cytidine deaminase activities that modify RNA or DNA. A3G exhibit varying degrees of inhibitory activity against HIV. In the absence of the Vif, A3G are packaged into HIV-1 particles through an interaction with Gag protein molecules and the help of cellular 7SL RNA and/or viral genomic RNA. Virion-packaged A3G mediates cytidine deamination in the viral minus-strand DNA during new target cell infection. Virion-packaged A3G can also reduce the accumulation of viral DNA by inhibiting reverse transcription processes or inducing viral DNA degradation. HIV-1 Vif suppresses the activity of A3G by assembling a viral-specific E3 ubiquitin ligase through its interaction with cellular Cullin5-ElonginB-ElonginC. Vif induces polyubiquitination of A3G and tags them for proteasome-mediated degradation. We thought that heat shock protein 70 (Hsp70) would have the action which protects A3G. Hsp70 family members facilitate assembly and disassembly of oligomeric protein complexes as well as their folding and intracellular transport. Hsp70 as a constituent of the HIV-1 virion and shown that the HIV-1 Gag polyprotein is sufficient for Hsp70 incorporation. There, it studies for the purpose of the elucidation the action of Hsp70 to the anti-HIV-1 activity of A3G. As a result, it was shown clearly that Hsp70 and A3G, and HIV-1 Gag are observed localization interaction with cytoplasm. Further, the amount of A3G incorporation into HIV-1 virions increased when Hsp70 was overexpressed. Thereby, the infectivity of HIV-1 declined. Showing possibility that over expression of Hsp70 could lead to inhibition of HIV-1 and we can suggest the possibility of HIV-1 therapy.

doi:10.1016/j.antiviral.2009.02.040

36

Immediate and Persistent Anti-HIV-1 Activity of the Biguanide-based Compound NB325 Involves Specific Interactions with the Viral Co-receptor CXCR4

Nina Thakkar^{1,*}, Vanessa Pirrone¹, Shendra Passic¹, Mohamed Labib², Robert Rando², Brian Wigdahl¹, Fred Krebs¹

¹ Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, USA; ² Novaflux Biosciences, Inc., Princeton, USA

Experiments designed to determine the mechanism by which NB325 (also known as polyethylene hexamethylene biguanide or PEHMB) inhibited HIV-1 infection suggested the involvement of cell surface molecules that participate in HIV-1 attachment and entry. In experiments involving flow cytometry, antibodies with distinct epitope specificities were used to detect CXCR4 at the cell surface and internalization induced by NB325 exposure. Competitive binding assays with the CXCR4 ligand, CXCL12, and peptides

derived from extracellular domains of CXCR4 were used to examine interaction specificity. Inhibition of CXCR4-induced chemotaxis was also assessed. NB325 altered the detection of CXCR4 on primary human CD45RO+CD4+ T lymphocytes in an epitope-specific manner. These results were consistent with the lack of inhibition of CXCL12 binding, potent inhibition of CXCL12-induced chemotaxis, and the lack of CXCR4 internalization after NB325 exposure. Peptide competition experiments supported the hypothesis that NB325 interacts with CXCR4 extracellular loop 2 (ECL2). Additional mechanistic experiments were performed to support the observation that NB325 provides antiviral memory, which persistently protects cells from subsequent HIV-1 infection. Experiments demonstrated decreased detection of CXCR4 ECL2 up to 24 h after removal of NB325 from the media, as well as reductions in CXCL12-induced chemotaxis after pre-exposure of cells to NB325. These results indicate that NB325 inhibits X4 HIV-1 infection and provides persistent protection from infection by a direct and specific interaction with CXCR4. These results are being used to guide studies designed to reveal the specific mechanism of action by which NB325 inhibits HIV-1 infection through the co-receptor molecule CCR5.

doi:10.1016/j.antiviral.2009.02.041

37

From β -Amino- γ -Sultone to New Bicyclic Pyridine and Pyrazine Heterocyclic Systems: Discovery of a Novel Class of HIV-1 Non-nucleoside Inhibitors

Sonsoles Velazquez^{1,*}, Sonia De Castro¹, Jan Balzarini², María-Jose Camarasa¹

¹ Instituto de Química Médica (C.S.I.C.), 28006 Madrid, Spain; ² Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Sultones are versatile heterocyclic intermediates whose chemistry, industrial applications and biological properties continue to be of interest. Even though recent chemical literature on saturated γ -sultones has been described (Enders and Iffland, 2007), research on the chemistry of α,β -unsaturated- γ -sultones (Braverman et al., 2007), and particularly of β -amino-substituted representatives of this family, is much less intensive. We recently described (de Castro et al., 2008) the reactivity of the 4-amino-5H-1,2-oxathiole-2,2-dioxide (β -amino- γ -sultone) heterocyclic system towards electrophiles and amines on readily available model substrates. One of the interesting features of the system is their ambident nucleophilicity; nucleophilic reaction can take place either at the site of the enamino carbon (C-3) or at the primary amino nitrogen depending on the nature of the electrophile and the reaction conditions (de Castro et al., 2008). We now explore the synthetic usefulness of this ambident nucleophile for the preparation of unusual fused nitrogen heterocyclic systems containing a γ -sultone moiety. In spite of the poor nucleophilicity of the amino group, which is considered to have an "amide like" character, the β -amino- γ -sultone system reacts with a variety of bis-electrophilic reagents to give previously unknown pyridine- and pyrazine-based bicyclic heterocyclic systems. The synthetic approaches for the preparation of these novel heterocycles and their biological evaluation against a broad panel of viruses in cell culture will be described. Interestingly, some substituted pyrido fused compounds showed a significant activity against HIV-1 infection, being inactive against a variety of other DNA and RNA viruses. The new "hit" compounds are an excellent starting point for the exploration of a novel class of HIV-1 non-nucleoside inhibitors.