31

Intermolecular Interactions of the $Pr55^{gag}$ Polyprotein and the Processed Matrix and Capsid Proteins of HIV-1

A.-D. Ellenrieder, R. Böttcher, B. Kattenbeck, H. Wolf and S. Modrow
Institute for Medical Microbiology and Hygiene, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

The $Pr55^{gag}$ polyprotein of HIV-1 plays a critical role during budding and formation of infectious particles. The HIV-1 matrix protein p17 as part of $Pr55^{gag}$ initially directs membrane localization of the assembling particle and forms an icosahedral shell associated with the inner side of the virus membrane. The processed p24 forms the conical core of mature HIV-1. Previously, we demonstrated that *in vitro* mutagenesis of HIV-provirus substituting alanines for amino acid triplets in p17 (AA 47 to 61: NPG LLE TSE GCR QIL) and in p24 (AA 341 to 352: ATL EEM MTA CQG) of $Pr55^{gag}$ inhibited virus assembly and infectivity. To get more information about intermolecular interactions between both the $Pr55^{gag}$ precursor and processed p17 and p24, we established a two-hybrid system in *S. cerevisiae* using plasmids that separately express a *lexA* DNA-binding and activation domains fused to sequences encoding $Pr55^{gag}$,p17, p24 or subfragments. Coexpression of the two fusion proteins results in the association of the *lexA* domains and potent activation of the *lexA*-controlled *β-gal* gene. Using $Pr55^{gag}$ mutated in p17 or p24 encoding sequences as fusion partner differences in the β-Gal expression could be demonstrated indicating influences on intermolecular protein interactions. In contrast processed forms of the p17, p24 and the C-terminal domain of p24 interact neither with each other nor with $Pr55^{gag}$. The results of these two-hybrid experiments suggest that the processed p17 and p24 proteins of HIV-1 do not have direct influence on protein-protein interactions, but play a critical role in the complex mechanisms of virus assembly mediated by p17 and p24 portions as part of $Pr55^{gag}$. Detailed knowledge of the underlying mechanisms will enable the development of new therapeutic agents.

32

The Activity of Mg^{2+} and Poly r(A-U) against HIV-1

M. R. Nassiri[1], J. M. Jamison[2], J. Gilloteaux[3], C.-c. Tsai[4] and J. L. Summers[2]

Departments of Pharmacology/Virology[1] and Anatomy[3], Lake Erie Coll. Of Osteopathic Medicine, 1858 W. Grandview Blvd, Erie, PA 16509, Department of Urology[2], Summa Health System/NE Ohio Univ Coll. Of Medicine, St. Rt. 44, Rootstown, OH 44272, Department of Chemistry[4], Kent State Univ., Kent, OH 44272, USA

Successful antiviral therapy with nucleoside analogs has been hampered by their toxicity and by viral resistance. Polyribonucleotide analogs are novel HIV inhibitors whose antiviral activity has not been fully explored. In this study polyribonucleotides alone and in combination with Mg^{2+} were tested for their antiviral activity against HIV and VSV. When Mg^{2+} was preincubated with poly r(A-U) or poly r(G-C) and tested in a human foreskin vesicular stomatitis bioassay, the 50% effective doses decreased up to 10-fold. Polydeoxyribonucleotides (PDN) alone, Mg^{2+} alone and the Mg^{2+} /PDN combinations were not efficacious antiviral agents. The enhanced antiviral activity was not due to increased interferon production of direc5t viral inactivation and no host cell toxicity was observed. A p24 ELISA assay system demonstrated that Mg2+ and the Mg2+/poly r(A-U) combination exhibit potent activity against HIV-1 3B infected peripheral blood mononuclear cells. Since phase contrast micrographs indicate the Mg2=/poly r(A-U) combinations localize in the nucleoli and chromatin of the host cells modulation of nuclear (nucleolar) processes may be responsible for the enhanced antiviral activity. Because the Mg2+/polyribonucleotide combinations are efficacious against both viruses, the clinical efficacy of this novel combination should be explored further.

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33

Temacrazine Inhibits Initiation of HIV-1 Transcription

J. A. TURPIN[1],* L. PALLANSCH[1], C. MICHEJDA[2], W. C. CHLODY[2], W. G. RICE[3] and R. W. BUCKHEIT, JR.[1], [1]Southern Research Institute, [3] Achillion Pharmaceuticals, Frederick, MD 21701 [2] ABL Basic Research, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21702.

Background: Temacrazine (1,4-bis[3-[(6-oxo-6H-v-triazolo[4,5,1-de}acridin-5-yl)aminopropyl]piperzine (NSC 687025)) is a novel nanomolar inhibitor of HIV-1 replication in acutely, chronically and latently infected cells. Mechanism of action studies suggested that it selectively inhibits HIV-1 transcription, although it is a potent inhibitor of HIV-1 integrase.

Methods: Inhibition of HIV-1 transcription was assessed using nuclear run-on assays to measure initiation and elongation of nascent RNA transcripts in chronically infected H9 HIV IIIB cells. Resistant virus was generated by serial passage of HIV-1 RF in CEM-SS cells in escalating doses of temacrazine. Complete resistance was achieved by 6 passages.

Results: Preliminary studies using nested PCR to detect integrated provirus showed that temacrazine did not inhibit HIV-1 integration. Temacrazine reduced RNA transcript initiation, but failed to effect elongation when measured in nuclear run-on assays. No effect on initiation or elongation of β-action or GAPDH transcripts was observed. Analysis of temacrazine resistant virus identified several unique nucleotide changes in the HIV-1 LTR at −1 (C→ A), −2 (T→A) and +111 (C →T) from the start of transcription. Mutations were also identified in integrase, but these changes were present in subtype F viruses, which maintained sensitivity to temacrazine.

Conclusion: Temacrazine selectively inhibits initiation of HIV-1 transcription by an unknown mechanism involving the HIV-1 LTR.

34

A Highly Potent NNRTI Possessing Potential Dual Activity Against HIV-1 Reverse Transcriptase and a Post-Attachment Intermediate.

C. Lackman-Smith[1], K. Watson[1], J.A. Turpin[1], L.A. Pallansch[1], S. Kodihalli[1], S.-G. Chung[2], E.-H. Cho[2], and R.W. Buckheit, Jr.[1] [1]Serquest-Southern Research Institute, Frederick, Maryland, USA and [2]Samjin Pharmaceutical Co., Ltd., Seoul, Korea

Recently a highly potent HIV-1 NNRTI, SJ-3366 (Samjin Pharmaceutical Co., Ltd., Seoul, Korea), was shown to have potent antiviral activity against HIV-1 (EC_{50} = 0.1 nM) with a therapeutic index in excess of 4 million. Interestingly, and unlike other NNRTIs, in addition to inhibiting reverse transcriptase, SJ-3366 also inhibited HIV-1 infection at a stage that precedes reverse transcription. Initial observations suggested that SJ-3366 was a potent inhibitor of virus attachment, but not virus cell fusion. Further experiments utilizing various specific chemokine receptor-expressing cell lines showed that SJ-3366 did not inhibit the interaction of gp120/CD4, nor was it virucidal. Additional studies suggested that it may interfere with HIV-1 entry via an intermediate target formed after virus-cell attachment has occurred. Data obtained utilizing a pseudotyped Vessicular Stomatitis Virus that expresses the HIV envelope will be presented to further characterize the mechanism of action of this novel, potent compound.