neurons against toxic substances in CSF from human infected patients with moderate to severe dementia. Mitochondrial potential was used to assess neuronal functionality. Didox nearly prevented the toxic CSF effect from moderate-severe dementia HIV patients on the mitochondrial potential of cultured fetal neurons.

These results demonstrate that this series of RRIs, particularly Didox, have multi-faceted actions that can be beneficial to HIV patients. They can impair HIV replication through inhibiting proviral DNA synthesis and potentiate NRTIs. Additionally, these RRIs can inhibit NF-kB activation. Lastly, they have the potential to impede the development of HIV dementia.

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Human Immunodeficiency Virus Type 1 Does Not Escape from Novel Single-Stranded Dnazyme Expression-Mediated Inhibition

Masaak Hyafune[1,*], Yuka Mouri[1], Naoko Miyano-Kurosaki[1,2], Kahoko Hashimoto[1], Hiroshi Takaku[1,2]

[1] Department of Life and Environmental Sciences, Japan; [2] High Technology Research Center, Chiba Institute of Technology, Tsudanuma, Narshino, Chiba 275-0016, Japan

Recently, several groups reported that the antiviral activity of shRNA targeting the HIV-1 gene is abolished due to the emergence of viral quasispecies harboring a point mutation in the shRNA target region. This finding is particularly relevant for viruses that exhibit significant genetic variation due to error-prone replication machinery, and the risk might be more severe for RNA viruses and retroviruses than for DNA viruses. On the other hand, ribozyme technologies are also major tools for inactivating genes in gene therapy. One model, termed deoxyribozyme (Dz), is especially useful because it can bind and cleave any single-stranded RNA at purine/pyrimidine junctions. The DNAzyme is similar to hammerhead ribozymes, at least in terms of its secondary structure, with two binding arms and a catalytic loop that captures the indispensable catalytic metal ions. We designed a vector to produce single-stranded DNA. Human inmmunodeficiency virus type 1 (HIV-1) reverse transcription was used to construct a DNAzyme expression vector against the HIV-1 env V3 loop (Kusunoki et al., 2003). Initiation of HIV-1 reverse transcription requires the formation of a complex containing the viral RNA, tRNALys-3, and reverse transcriptase. The expression vector contains the HIV-1 primer binding site and tRNALys-3 at the 3′ end of its RNA transcript, thus enabling the synthesis of a single-stranded DNA by HIV-1 reverse transcriptase. We demonstrated that the lentiviral vector-mediated DNAzyme expression suppressed HIV-1 replication in SupT1 cells. Furthermore, HIV-1 did not escape from novel single-stranded DNAzyme expression-mediated inhibition. This lentiviral vector-mediated DNAzyme anti-genes are promising tools for HIV-1 gene therapy for the treatment of HIV/AIDS.

Reference

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Characterization of a New Class of Polycyclic RSV Inhibitors

Angela Luttick[1,*], Bo Lin[1], Craig Morton[1], Simon Tucker[1], Silas Bond[1], Alistair Draffan[1], John Lambert[1], Chin-Yu Lim[1], Jeff Mitchell[1], Vanessa Sanford[1], Mike McCarthy[2], JoAnn Suzich[2], Nita Patel[2], Bettina Richter[2]

[1] Biota Holdings Limited, Australia; [2] MedImmune Inc., USA

Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia in children under one year of age and is a leading cause of severe lower respiratory infections in infants and young children. Prophylactic antibodies such as Synagis® (palivizumab) effectively reduce the incidence and severity of RSV disease in high-risk pediatric populations but the only antiviral treatment available for patients with RSV disease is ribavirin, a nucleoside analog with suboptimal clinical efficacy and safety profile.

RSV enters cells in the lung using a fusion glycoprotein (RSV-F), found on the virus's outer envelope. Biota has developed small-molecule, orally available, synthetic drugs that specifically target RSV-F, preventing it from functioning and therefore stopping RSV infection from spreading.

We will present in vitro cellular data evaluating the antiviral activity and cytotoxicity of this potent class of RSV inhibitors. Mechanism of action will be reported including functional assays and genotypic and phenotypic analysis of resistant mutants. Cross-resistance data with known fusion inhibitors and modelling studies to establish the proposed binding site will be presented. The compounds display promising oral bioavailability and efficacy in rodent models of RSV infection.

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HIV Coreceptor Switch Induced by Antagonism to CCR5

Gemma Moncunill*, Mercedes Armand-Ugón, Bonaventura Clotet, José A. Esté

Retrovirology Laboratory irsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, 08916 Badalona, Spain

HIV resistance to CCR5 antagonists in cell culture has been observed in the absence of coreceptor switch, but it is unclear whether inhibition of HIV-1 replication with a CCR5 antagonist will lead to an increased rate of emergence of CXCR4 variants.

R5 HIV-1 strains were cultured in lymphoid cells expressing high levels of CXCR4 and low levels of CCR5 in the absence or presence of agents targeting CXCR4 (AMD3100), CCR5 (Tak-779, mAb 2D7 and RANTES) and the reverse transcriptase (AZT). Cell cultures were kept for up to 200 days. Syncytium formation and virus replication (p24 antigen in the supernatant) were recorded weekly. Viral coreceptor use was evaluated in MT-2 cells and U87-CD4+CCR5+/CXCR4+ cells at different times. Proviral DNA of selected passages was used to identify amino acid changes in env. Virus replication was reminiscent of slow replicating, R5 phenotype but could be blocked by Tak-779, RANTES or mAb 2D7. One of the six strains was resistant to AZT. In the absence of drug pressure, three out six strains used were able to switch from the R5 to the X4 phenotype and showed increased replication rate. Coreceptor switch could be delayed by AZT or Tak-779 in all three strains. However, under similar drug-pressure, outgrowth of virus could be detected faster in the presence of CCR5 inhibitors but not in the presence of AZT and was concomitant to its ability to use CXCR4. Coreceptor switch was noticed earlier in the presence of AZT than Tak-779 in the culture of the AZT-resistant virus. Conversely, treatment with AMD3100 prevented the emergence of CXCR4-using virus. In conclusion, under our experimental conditions, a lower replication rate (i.e. AZT or Tak-779 versus no drug treatment) slows coreceptor switch. However, under similar replication conditions (i.e. AZT versus Tak-779) CCR5 drug pressure may induce a faster emergence of CXCR4-using HIV. A cell culture model of the evolution of HIV-1 coreceptor use may be relevant to assess the propensity of clinical isolates to develop drug resistance through a change in virus coreceptor.

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Induction of IL-6 and IL-8 by siRNAs Targeting HIV Coreceptor CCR5

Eduardo Pauls*, Jordi Senserrich, Margarita Bofill, Bonaventura Clotet, Jose Esté

Retrovirology Laboratory irsiCaixa, Spain

The HIV-1 coreceptor CCR5 has been thought a relevant target for small interfering RNA (siRNA)-based therapeutics. However, recent findings suggest that siRNA can stimulate innate cytokine responses in mammals. All siRNA agents tested were able to down-regulate the expression of CCR5 albeit with different efficiency (51-74% downregulation), block HIV induced syncytia formation between HIV-1 BaL-infected and uninfected CD4+ cells or block single round HIV-1 infection as measured by a luciferase reporter assay (46-83% inhibition). Conversely, siRNA directed against CCR5 did not affect replication of a VSV pseudotyped virus, suggesting that inhibition of HIV replication was specific to CCR5 down-regulation. However, 2 of 4 siRNA tested were able to induce the production of interleukin-6 (6-fold induction) and interleukin-8 (9-fold induction) but no IFN-(, IFN-(, IFN-(, TNF-(, MCP-1, MIP-1(, MIP-1(, RANTES, IL-1(, IL-10 or IL-12p70 cytokine induction was noted. In the absence of detectable IFN-(, IL-6 or IL-8 may represent markers of non-specific effect triggered by siRNA.

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Identification and Characterization of a Novel, Potent HCV Helicase Inhibitor

Junzhong Peng*, Chunsheng Huang, Michael G. Murray, Zhuhui Huang

Southern Research Institute, Frederick, MD 21701, USA

Hepatitis C virus (HCV) is the leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. Current interferon-based standard of care has demonstrated limited success and is associated with undesirable side effects. Significant efforts have been directed towards the discovery and development of effective therapeutics to treat HCV infections and patients relapsing from interferon therapy. We undertook the task to screen a compound library for anti-HCV activity using the subgenomic HCV replicon-based reporter system. Trioxsalen was identified as a potent HCV inhibitor. Trioxsalen belongs to the group of medicines called psoralens, and is an FDA approved drug used along with ultraviolet light to treat vitiligo. The compound suppressed replication of the HCV replicon in a dose-dependent manner with a EC_{50} value of 1.8 μM. Interestingly, trioxsalen had no antiviral activity against both BVDV and YFV, members of flaviviruses related to HCV and two trioxsalen analogs, 5-methoxypsoralen and 8-methoxypsoralen, did not display any inhibition in the HCV replicon system. In order to determine the mechanism of action of trioxsalen, the compound was examined for inhibition of the HCV NS5B polymerase, the NS3 helicase and the NS3/4A proteinase. The results demonstrated that trioxsalen did not exhibit appreciable inhibitory effects on the NS5B polymerase activity and the NS3/4A proteinase activity; however, the compound acted as a potent inhibitor against the helicase activity by inhibiting the unwinding process. As the helicase is crucial for the HCV life cycle, trioxsalen may represent a novel class of therapeutics to treat HCV infections.

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