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Antiviral Activity of the MEK-inhibitor U0126 against Pandemic H1N1v and Highly Pathogenic Avian Influenza Virus *In Vitro* and *In Vivo*

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Zoonotic, seasonal epidemic and pandemic Influenza is still a formidable foe throughout the world. The emergence of the 2009 H1N1 pandemic swine influenza A virus is a good example on how this viral infection can impact health systems around the world. The continuous zoonotic circulation and reassortment of influenza viruses in nature represents an enormous public health threat to humans. Besides vaccination antivirals are required to efficiently control the disease. In the present work we investigated that the MEK inhibitor U0126, targeting the intracellular Raf/MEK/ERK signalling pathway, reduces the 2009 pandemic influenza strain H1N1v and highly pathogenic avian influenza viruses in cell culture and in the mouse lung. U0126 showed antiviral activity in cell culture against all tested influenza virus strains including oseltamivir resistant strains. We were able to demonstrate that treatment of mice with U0126 via the aerosol route led to (i) inhibition of MEK activation in the lung, (ii) reduction of influenza virus titer compared to untreated controls, (iii) protection of influenza virus infected and U0126 treated mice against a 10× lethal challenge. Moreover, no adverse effects of U0126 were found in cell culture or in the mouse. Thus the principal conclusions of our findings are that U0126 inhibiting the cellular target MEK has an antiviral potential in cell culture and in the mouse model. Since MEK inhibitors are tested in various clinical trials against cancer, indicating that MEK inhibitors might be safe and well tolerated.

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Targeted Elimination of HIV Infected Cells: Synergistic Combination of Dexamethasone and DEAE as a Paradigm

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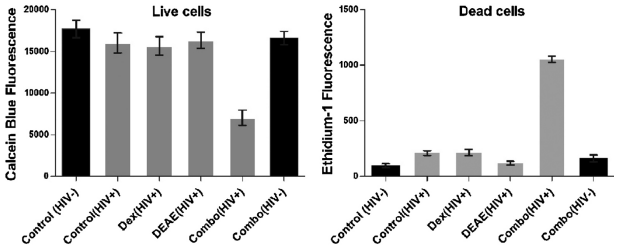
Highly Active Antiretroviral Therapy (HAART) has proven to be successful in controlling HIV infection, yet it remains incapable of eradicating the virus. Systemic infection re-emerges upon treatment interruption, requiring a lifetime of costly drug therapy with an ever-present risk of emerging drug resistance. Thus, there is a critical need for the development of new treatment modalities not based solely on virally encoded targets, and with the potential to actually cure HIV infection. Our research introduces the innovative concept of a small molecule drug regimen that has the ability to selectively eliminate HIV infected cells from the body. Using standard colorimetric and fluorometric "Live/Dead" cell labeling techniques, we have developed an easily automated "selective cell death" (SCD) assay, and identified a lead drug combination for the elimination of infected cells, involving a patented combination of two generic FDA approved drugs or their metabolites: the glucocorticoid dexamethasone (Dex) and *N*,*N*-diethylaminoethanol (DEAE), a metabolite of procaine.

As shown in the figure, neither drug alone, but only the combination (Combo), is able to induce cell death, and only in HIV-infected cell cultures ($P < 0.0001$). Prolonged exposure of cells to this drug combination leads to a decline in viral load: two weeks of treatment resulted in a decrease of more than 50% in viral titer relative to untreated control cells. These results provide proof of concept for the utility of our SCD assay, and the ability to identify small molecule drug combinations that can selectively kill HIV-infected cells. Such therapies would complement and enhance the effectiveness of standard antiretroviral drug regimens that inhibit HIV replication.



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Design and Synthesis of New Isatin Derivatives as HIV-1 Reverse Transcriptase Associated Ribonuclease H Inhibitors

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The human immunodeficiency virus (HIV) is the etiological agent of the acquired immunodeficiency syndrome (AIDS) in humans. Despite the fact that many therapeutic agents targeted to the viral reverse transcriptase (RT), the multifunctional enzyme which is responsible for the viral genome replication, are already clinically available, none of them is active on the RT-associated Ribonuclease H (RNase H) activity, whose function is essential for viral replication and, hence, is an attractive target for drug development. In the last few years, a few classes of compounds have been identified as HIV-1 RNase H inhibitors, however, none of them was able to reach clinical trial testing. Thus, efforts in the development of new compounds targeting the RNase H activity are relevant to enhance the antiretroviral armamentarium and constitute an attractive challenge for medicinal chemists. In this perspective, within an RNase H drug discovery program, we designed and synthesized a series of differently substituted isatin derivatives and tested them on both HIV-1 RT-associated RNase H and DNA polymerase functions. Within these compounds, isatin derivatives appeared as promising scaffold for the inhibition of the HIV-1 RT-associated RNase H activity. The resulting SAR study may provide significant hints for the determination of the pharmacophoric requirements for the interaction with this viral target.

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