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NAC Reduces Apoptosis and Telomeres Shortening Subsequent to HIV-1 Exposure in an Astrocytoma Cell Line

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Oxidative stress, involved in HIV-1 disease, plays a key role in the neuropathogenesis of HIV-1 infection. HIV-1 infected cells produce free radicals, involved in the apoptosis of astroglia and neurons. Recent data show that oxidative stress is responsible also of accelerates telomere shortening of human fibroblast in vitro. Our study was focused on the relationship between HIV-1/oxidative stress/astrocytic damage. U373 human astrocytoma cells were directly exposed to X4-using strain HIV-1_{IIIB}, for 1, 3, 5, 10 and 15 days and treated (where requested) with different doses of *N*-acetylcysteine (NAC), compound essential for the synthesis of glutathione (GSH), a cellular antioxidant. Apoptosis was analyzed by FACS analysis, and telomere length, by Quantitative-FISH (Q-FISH). Intracellular GSH and GSSG were determined by high-performance liquid chromatography (HPLC). Statistical analysis was performed by χ^2 test ($p < 0.001$). Incubation of U373 with HIV-1_{IIIB} led to significant induction of cellular apoptosis (1 day: 17%; 3 days: 32%; 5 days: 70%; 10 days: 54%; 15 days: 76%). Apoptosis was reduced of 48% in the presence of 1mM NAC at day 5 after virus exposure ($p < 0.001$). Moreover, NAC improved the GSH/GSSG ratio, a sensitive indicator of oxidative stress that decreased strongly after HIV-1_{IIIB} exposure in U373. Analysis of telomere length showed, in HIV-1 exposed U373, a statistically significant telomere shortening (1 day: 18%; 3 days: 11%; 5 days: 55%), that was completely resumed in U373 NAC-treated. Our results support the role of HIV-1-mediated oxidative stress in astrocytic death, and the importance of antioxidant compounds in preventing these cellular damages. Moreover, indicate that the telomere structure, target for oxidative damage, could be the key sensor of cell apoptosis induced by oxidative stress after HIV infection.

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A Novel NNRTI Class with Potent Anti-HIV Activity Against NNRTI-resistant Viruses

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Background: Current highly active anti-retroviral therapy (HAART) strategies for HIV infection utilize combinations of at least two classes of anti-retroviral agents. Although HAART has proven to be effective, its benefits can be compromised by the development of drug resistance. For the NNRTIs, many mutations such as K103N cause cross-resistance, rendering this class unavailable for combination therapies in subjects infected with these resistant viruses. Newer NNRTIs, active against the NNRTI-resistant viruses, are urgently needed to expand the NNRTI armamentarium. The characterization of activities against a panel of NNRTI-resistant HIV-1 viruses suggests this NNRTI series has the potential to overcome the most prevalent of these resistant strains.
Methods: Antiviral activities of the NNRTIs were determined using either VSV-g pseudotyped HIV-1 containing wild type (wt) and NNRTI-resistant sequences or clinical HIV-1 isolates containing NNRTI-resistant mutations. Cytotoxicity was evaluated in primary human cells and cell lines. Non-linear regression analysis was used to calculate IC_{50} values.
Results: The NNRTI series comprises potent inhibitors of wt HIV-1 with EC_{50} values of approximately 1 nM and CC_{50} values of >50 μM. The fold changes (FCs) in EC_{50} against the major NNRTI-resistant viruses found in patients failing efavirenz therapy are significantly lower than those of efavirenz. For instance, FCs for compounds in the series against K103N are <1, versus >10 for efavirenz. The FC in activity over wild type virus in a broad panel of NNRTI-resistant mutant viruses is superior to efavirenz and superior or similar to the TMC NNRTIs in development. These compounds are stable in human plasma.
Conclusions: Compounds in this series are potent NNRTIs with a large selectivity index. They are superior to efavirenz against a broad panel of NNRTI resistant viruses. The in vitro characterization of these novel NNRTIs shows strong potential for improved performance over current NNRTIs and warrants further evaluation.

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A Recombinant, Infectious Human Parainfluenza Virus Type 3 Expressing the Enhanced Green Fluorescent Protein for Use in High Throughput Antiviral Assays

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For some viruses, antiviral assays are quite tedious, either in the duration of the assay or the difficulty of growth in cell culture. The ability to rescue an infectious, RNA virus from a cDNA clone has led to new opportunities for

detecting viral expressed reporter genes to measure viral expression. In this case, the enhanced green fluorescent protein was inserted into the human parainfluenza virus type 3 genome. The rescued, recombinant virus (rHPIV3-EGFP) was plaque purified three times in MA104 cells and titered, by plaque assay, to $1.9 \times 10^7 \pm 4.9 \times 10^6$ PFU/mL, whereas the wild-type 14702 strain (HPIV-3 WT) was titered to $2.9 \times 10^7 \pm 4.1 \times 10^6$ PFU/mL. 50% efficacy values were calculated from neutral red-based antiviral assays with rHPIV3-EGFP and HPIV-3 WT viruses using ribavirin (16 ± 0.58 μg/mL and 35 ± 2.5 μg/mL, respectively) and 2-thio-6-azauridine (0.63 ± 0.075 μg/mL and 1.5 ± 0.2 μg/mL, respectively). Cytopathic effect (CPE) of each virus was measured by neutral red over a 7-day period; no differences were seen. Even though the plaque titers and antiviral data suggest a slightly attenuated EGFP virus, the CPE data suggests that *in vitro* differences are minimal. To determine when maximum GFP levels were obtained, rHPIV3-EGFP was infected at various MOIs and fluorescence was read each day for 8 days. GFP expression reached its maximum at day 3 in a dose responsive manner. Comparison of a 3-day GFP-based antiviral assay to a 7-day neutral-based antiviral assay yielded Z′-factor values of 0.83 and 0.70, respectively, signal-to-background ratios of 241 and 65, respectively, and signal-to-noise ratios of 4057 and 301, respectively. These data suggest the superiority of the GFP-based antiviral assay to the neutral red-based assay. A 3-day GFP-based assay and a 7-day neutral red-based assay were run side-by-side and significant indexes (SI) were calculated. Using a SI threshold of 10, the GFP-based antiviral assay had a sensitivity of 89% and a specificity of 47%. The use of a GFP-based antiviral assay for testing potential antiviral compounds against HPIV-3 in a high throughput format has been justified for initial screening purposes.

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NGO Analysis of Impact of HIV Infection and Antiretroviral Therapies in Resource Poor Nations Are We on Right Path to Control HIV

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Objectives: AIDS activists have argued against poor availability, high-cost of ARV drugs. Past studies proved that HAART non-cost-effective outside industrialized nations. Our Indian NGO explored/analyzed economic impact of HIV infection and antiretroviral therapies in rural/tribal communities. Building strategic alliances with NGOs and government agencies to provide discounted ARV's is propogated.

Methods: Analysis of available 16 studies on HIV-Rx cost, WHO publications/declarations by IAS/UNAIDS/global-AIDS fund. Time frame for statistical analysis taken from April 2004 till todate. Nine corporate, seven NGO and five Govt-sector programs undertaken for review.

Results: NGOs had limited opportunity to learn about HIV best practice and acquire technical skills and develop organizational capacity. Poor institutional infrastructure/systems to support program implementation raises questions about effective ways to develop community-base responses. Factors like Percapita income, social-standing change outcome of HIV drug therapy/compliance. Economic effects included low productivity, increased medical consultations/hospitalizations. Average cost of ARV therapy in subsidized centre US$ 1200 Vs opportunistic infections US$ 1385 per patient for 1 month. Additional costs for nutrition, supportive therapy/palliative care comes to US$ 800. Considering family income at US$ 400 in rural India, current HIV therapy is out of reach for >84% population.

Conclusion: HIV policy makers must form collaborative efforts to reduce cost, increase access to ARV drugs. Community NGO representatives must be made part of decision-making body of IAVI/IAS/UNAIDS. Realizing divergent versions of cost analysis a multicentre study on this burning issue be carried out in developing nations. ARV-drug development is a long-term commitment that must also consider financial constraints of population from south. At 21st ICAR-2008 conference, we shall form group of NGO activists and researchers from USA/Europe to substantially improve Anti-Retroviral-Drug-service provision policy.

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A Cell-based High-throughput Screening Approach for the Discovery of New Inhibitors of the Influenza H5N1 Virus

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Using a highly reproducible and robust cell-based HTS assay, we screened the NIH Molecular Libraries Screening Centers Network (MLSCN) 100,000 compound library at 50 μM compound concentration against influenza strain AV/VN/1203/2004 (H5N1). The "hit" rate (>25% inhibition of the viral cytopathic effect) from the single dose screen was 0.32%. The hits were evaluated for their antiviral activity, cell toxicity and selectivity in dose response experiments. The screen yielded five active compounds (SI_{50} value > 3). One compound showed an SI_{50} value of greater than 3.45, three compounds had SI_{50} values ranging from greater than 13.84 to 34.29, while the most active compound displayed an SI_{50} value of 94.64. The active compounds represent two different classes of molecules, benzoquinazolinones and thiazoloimidazoles which have not been previously identified as having anti-viral/anti-influenza activity.

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