37

Antiretroviral activity and selective cytotoxicity of Lithium gamma-linolenic acid (LiGLA). S.I. Randall (1), D. Kinchington (1), M.D. Winther (2), D.F. Horrobin (2), W.F. Schlech (3), D. Kumar (4), O. Mpanju (4), B. Conway (4). Department of Virology, St-Bartholemew Medical College, London, UK (1); Scotia Pharmaceuticals, Nova Scotia, CAN (2); Dalhousie University, Halifax, CAN (3); University of Ottawa AIDS Research Group, Ottawa, CAN (4).

The cytotoxicity of LiGLA was assessed in H9 cells chronically infected with HIV-1 RF (H9-RF cells). After 4-5 days exposure to 20μg/ml LiGLA, 90% of cells were non-viable (vs 20% for H9 control cells cultured under the same conditions). Pre-treatment of H9 cells with 10μg/ml LiGLA reduced syncitium formation on co-culture with H9-RF cells by 50%. Both of these effects were abolished by pre-treatment with the anti-oxidant vitamin E, suggesting that the observed effect may be due to enhanced lipid peroxidation of the drug in infected cells. In order to study this effect in a more clinically relevant cell type, we tested mononuclear cells (MCs) obtained from seronegative donors. This also enabled us to monitor specific antiretroviral activity. Initially, significant cytotoxicity was observed in PHA-stimulated MCs exposed to 10-30μg/ml LiGLA for 24 hours, then infected with HTLV-IIIB (MOI=0.01). These experiments were repeated, including incubation of uninfected cells with 10μg/ml LiGLA for 12 days (no cytotoxicity) and pre-incubation with drug for 1 or 4 days prior to infection with a lower titer of HTLV-IIIB (MOI=0.005). At doses below 20μg/ml LiGLA, no significant cytotoxicity was observed until 8 days following infection, independent of the total duration of exposure to LiGLA, suggesting a selective cytotoxicity for infected cells. Quantitative p24 antigen levels (ng/ml/10^6 cells) on days 4 and 8 post infection are shown below:

			LiGLA	
	Control	1μM AZT	10μg/ml	15μg/ml
Day 4	5.7	0.2	6.1	4.9
Day 8	83	0.1	21.4	3.8

This compound appears to have antiviral activity in this model at concentrations of 10-15μg/ml, although inferior to that of AZT. LiGLA will be the subject of more extensive *in vitro* studies as well as phase I trials in HIV-infected patients in Canada and the United Kingdom.

38

Biological and Biochemical Anti-HIV Activity of a Phosphorothioate G-Quartet (TTGGGGTT). R.W. Buckheit, Jr.[1], J.R. Roberson[1], J.R. Wyatt[2], T.A. Vickers[2], E. DeBaets[2], P.W. Davis[2] and D.J. Ecker[2], [1]Southern Research Institute-Frederick Research Center, Frederick, MD, USA, [2]ISIS Pharmaceuticals, Carlsbad, CA, USA

The phosphorothioate oligonucleotide T2G4T2 (ISIS 5320) was identified as a potent inhibitor of HIV infection *in vitro* by combinatorial screening of a library of phosphorothioate oligonucleotides that contained all possible 8-nucleotide sequences. The oligonucleotide forms a parallel-stranded tetrameric guanosine-quartet (G-quartet) structure which specifically binds to the HIV envelope glycoprotein (gp120) at the V3 loop and inhibits both cell-to-cell and virus-to-cell infection at submicromolar concentrations. The G-quartet inhibits the infection of laboratory derived isolates of HIV-1 and HIV-2 in a variety of tissue culture cell lines (CEM-SS, MT2, U937, AA5), inhibits a panel of diverse clinical isolates in fresh human peripheral blood lymphocytes and macrophages, and inhibits all drug-resistant virus isolates tested. In combination with AZT, ISIS 5320 exhibits additive to synergistic anti-HIV activity. Cell-based mechanism of action studies demonstrate that the compound inhibits the binding of infectious virus and virus-infected cells to uninfected target cells. The compound is stable in serum and preliminary data suggests favorable pharmacokinetics with micromolar concentrations sustained in the lymph nodes over a period of several days. The G-quartet compound is a new structured motif and a potential candidate for use in anti-HIV chemotherapy.