24

AZT TREATMENT OF HIV-1-INFECTED MACROPHAGES PREVENTS APOPTOSIS AND NECROSIS IN HUMAN ASTROCYTIC CELLS

CF. Perno, S. Aquaro, S. Panti, E. Balestra, MC. Caroleo*, A. Mastino°, A. Cenci and R. Calio
Department of Experimental Medicine, University of Rome "Tor Vergata", Italy *Chair of Pharmacology, University of Calabria, Cosenza, Italy and °Institute of Microbiology, University of Messina, Italy

Objectives of the study: Assess whether antiviral drugs are able to improve astrocytic damage induced by infection of macrophages with HIV. Primary macrophages obtained from seronegative donors were infected in vitro with HIV in the presence or absence of AZT. Astrocytic cells were then exposed to the supernatants of infected- or mock-infected macrophages. Their viability and levels of apoptosis were assessed by trypan blue exclusion, FACS analysis, and TUNEL staining. The supernatant of infected macrophages (but not that of macrophages not exposed to HIV) was able to induce a dramatic decrease of viability of astrocytes, starting from day 6 after exposure. This effect is not related to the infection of astrocytes by HIV, since both p24 (assessed at all time points of the experiment) and DNA-PCR were negative; in addition, CD4 expression was also negative. FACS analysis shows a very low viability of astrocytic cells (lower than 5% after 14 days of culture), that is not completely related to apoptosis. Indeed, both TUNEL staining and FACS analysis show apoptosis in about 40% of the astrocytic cells (compared to 9% in those exposed to mock-infected supernatants), while the other cells seem to be dying for necrosis. The phenomenon cannot be reproduced by exposing astrocytes to recombinant gp120 (the levels of apoptosis under these conditions were superimposable to those obtained in mock-infected exposed astrocytes). More important, the supernatants of macrophages infected but treated with AZT (with levels of p24 undetectable) were unable to induce neither apoptosis nor necrosis in astrocytes. Overall results may give some insights about the pathogenesis of the HIV-related encephalopathy, and more important, can contribute to the understanding of the mechanism(s) by which AZT potently reverses the damage of central nervous system typically found in patients infected by HIV.

25

ENHANCEMENT OF THE ION-PAIRING HPLC ANALYSIS OF CELLULAR 2'-DEOXYADENOSINE 5'-TRIPHOSPHATE, 2',3'-DIDEOXYADENOSINE 5'-TRIPHOSPHATE AND 2'-FLUORO-2',3'-DIDEOXYADENOSINE 5'-TRIPHOSPHATE POOLS THROUGH PERIODATE-TREATMENT AND FLUOROGENIC DERIVATIZATION

H.ZHANG*, B. HALL, S. KUNDER, O.L.WOOD, M. A. BACHO, H. FORD[1], S. PAPERMASTER, P. L. BLACK, C. NIELSEN AND M.A. USSERY. U.S. Food and Drug Administration, Rockville, Maryland 20857; [1]NCI, Bethesda, Maryland 20892

An assay for simultaneous quantitation of 2'-deoxyadenosine 5'-triphosphate (dATP), 2',3'-dideoxyadenosine 5'-triphosphate and/or 2'-fluoro-2',3'-dideoxyadenosine 5'-triphosphate within cultured murine cell line and human peripheral blood mononuclear cells (PBMC) exposed to ddI and/or F-ddA is described. Following destruction of ribonucleotides by treatment with periodate of the samples, derivatization coupled with fluorescence detection has been investigated as a means of enhancing sensitivity for the ion-pairing HPLC analysis of dATP, a natural occuring deoxynucleotide, and 2',3'-ddATP or 2', 3'-F-ddATP, active antiviral metabolites of ddI or F-ddA. The reaction of chloroacetaldehyde with the adenine base of dATP, and ddATP or F-ddATP has been employed to form fluorescent 1, N^6-etheno derivatives of dATP, and ddATP or F-ddATP. These derivatives are separated by ion-pairing HPLC using a C18 ODS column and give an analytically useful fluoresence emission at 403nm after excitation at 275 nm. Derivatization, fluoresence detection and chromatography have been optimized for the analysis of nanomolar concentrations of dATP, ddATP or F-ddATP within cells exposed to dideoxynucleosides. This technique is suitable for high sensitivity quantitation of dATP, and ddATP or F-ddATP pools in samples from preclinical and clinical pharmacology studies.

26

A Non-Human Primate Model for *In Vivo* Evaluation of Anti-HIV-1 Drugs. B. Rosenwirth, W.M.J.M. Bogers, K. Ueberla and J.L. Heeney, Biomedical Primate Research Center, 2288GJ Rijswijk, The Netherlands.

At present, heterosexual transmission accounts for up to 90% of new HIV infections worldwide. Therefore, it is of global importance to develop strategies to combat further spread of the epidemic via this route. Prophylactic treatment with antiviral drugs before or shortly after mucosal exposure may limit virus infection to the mucosa and the draining lymph nodes and/or may reduce virus load such that the immune system can eliminate the virus or, at least, keep it under long-lasting control. Anti-HIV chemotherapy so far has concentrated on the development of drugs to prevent or delay disease progression. Prophylactic clinical trials meet ethical, sociological and technical problems, therefore, an animal model should be used first. SIV infection of rhesus macaques allows infection via various routes, monitoring of virological and immunological parameters and observation of disease development. Prevention of SIV infection by an antiviral, PMPA, was recently demonstrated in the macaque model. PMPA is active against various retroviruses, other drugs, however, are highly specific for HIV-1. To test such substances in the macaque model chimeric viruses will have to be used, containing the HIV-1 specific gene for the target protein in an SIV genomic background. Such SHIV chimeras have been constructed recently. A chimera containing the HIV-1 RT gene in the genomic background of SIV has been reported to cause disease in macaques as did some of the HIV-1 envelope bearing SHIV. These SHIV strains now allow *in vivo* testing of substances being targeted selectively to, e.g., HIV-1 envelope or reverse transcriptase. We are presently selecting/constructing SHIV strains which effectively infect rhesus macaques via the mucosal route. The susceptibility of these strains to inhibition by selected antivirals is evaluated *in vitro* and *in vivo*. Virological and immunological parameters of SHIV infection of rhesus macaques are monitored in the mucosa, in the lymph nodes and in the periphery. This non-human primate model will serve to evaluate antivirals for their potential to be used in successful prophylaxis of HIV infection and disease.

27

Antiretroviral Activity and Metabolism of bis(POC)PMPA, An Oral Bioavailable Prodrug of PMPA. A. Fridland, B.L. Robbins, R.V. Srinivas, St. Jude Children's Research Hospital, Memphis, TN, USA; and M. Arimilli, C. Kim, N. Bischofberger, Gilead Sciences, Foster City, CA, USA.

PMPA is an acyclic nucleotide analog that has shown marked efficacy in the prevention of SIV infection in rhesus macaques. PMPA has also reduced SIV RNA by 2-3 logs in chronically infected macaques. To increase the low bioavailability of PMPA, a prodrug derivative bis(isopropyloxy carbonyl oxymethyl)PMPA was developed as a potential clinical candidate. Bis(POC)PMPA is chemically stable at low pH and has shown 30% bioavailability in dogs with minimal toxicity in repeat 5-day dose administrations of 60 mg/kg/day. The anti-HIV-1 activity of bis(POC)PMPA in freshly isolated human peripheral blood lymphocytes and in dendritic-T cells coculture system was 35- and 16-fold, respectively, greater than that of PMPA. Both PMPA and bis(POC) PMPA were virtually non-toxic at concentrations that completely suppressed viral replication. Studies of the metabolism of [^{3}H] bis(POC)PMPA showed that it was rapidly taken into the human cells, hydrolyzed to the parental PMPA, and phosphorylated to the mono- and diphosphate derivatives. By 60 min of incubation with one μM [^{3}H]bis(POC) PMPA, the concentration of PMPA, PMPAp and the active diphosphate, PMPApp, in the human cells was 28, 1.9 and 6.3 μM, respectively. By contrast, activation of free [^{3}H]PMPA in the human cells was virtually undetectable during this period. These results show that bis(POC)PMPA is a membrane permeable form of PMPA and shows promise as a drug for the treatment and prophylaxis of HIV infections.