54

Cell Type-Dependent Effect of Sodium Valproate on HIV-1 Replication *In Vitro*

M. Witvrouw, J.-C. Schmit, J.A. Esté, B. Van Remoortel, J. Desmyter and E. De Clercq
Rega Institute for Medical Research, K.U.Leuven, Leuven, Belgium

Sodium valproate (VPA), a simple, branched-chain fatty acid with a broad spectrum anticonvulsant activity is used in the treatment of many forms of epilepsy. Seizures are common in HIV-1-infected patients, either due to opportunistic infections (i.e. toxoplasmosis) and cancers (i.e. lymphoma) or to virus-related lesions of the brain (i.e. encephalopathy). These conditions frequently require anticonvulsant therapy, and VPA may be considered as a possible therapeutic choice. However, VPA has been reported to stimulate HIV-1 replication *in vitro* in acutely infected CEM and chronically infected U1 cells (Chemico-Biological Interactions 91: 111-121, 1994). We have now examined the effects of VPA on HIV replication in a variety of cell systems. We have found that VPA is able to stimulate HIV-1 replication in some cell systems but not in others. VPA stimulated HIV-1 replication in acutely infected CEM and C8166 T-lymphocytic cell lines and chronically infected ACH-2 and U937/III_B cells in a concentration-dependent manner. However, we could not detect any stimulatory effect of VPA on HIV replication in acutely infected peripheral blood mononuclear cells (PBMC), MT-4, MT-2, HUT-78 or MOLT-4 (clone 8) cells and in chronically infected HUT-78/III_B cells. The stimulatory effect of VPA on HIV-1 replication was noted at drug concentrations that were generally toxic to the host cells. The stimulatory effect shown by VPA under certain conditions may be ascribed to enhanced HIV transcription, as VPA was found to enhance (up to 2-fold) the HIV-long terminal repeat (LTR)-directed expression of β-galactosidase in transiently transfected HLtat cells. VPA had no effect on HIV-induced syncytium formation and did not show a direct virucidal action.

55

COMPARISON STUDY OF TRANSACTIVATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1)-LTR ON DIFFERENT CELL MODELS

O.A. Mamaeva, O.A. Plyasunova, N.M. Gashnikova, A.G. Pokrovsky, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Russia

Human tumor necrosis factor (α-TNF) play significant role in the HIV-infection pathogenesis, moreover as known it reverses the anti-HIV activity of AZT in cell culture. The search for the relationship between the recombinant α-TNF and transactivation of HIV-1-LTR has been made on two cell models: Jurkat-tat cells transient transfected with plasmid pHIVlacZ containing the HIV-1-LTR and reporter gene β-galactosidase and Jurkat-tat cells HIV-1_{EVK} infected. The Jurkat-tat cells were transfected with plasmid pHIVlacZ using the DEAE-dextran procedure. Expression of the β-galactosidase in cells was documented by ONPG. Jurkat-tat cells was infected with HIV-1_{EVK} using multiplicity of infection 0.2-0.5. Expression of the viral p24 protein was measured by ELISA method. It was found that 100 U/ml recombinant α-TNF stimulated the virus production in HIV-1 infected Jurkat-tat cells until 126%. The stimulation of β-galactosidase exspression in Jurkat-tat cells transfected with plasmid pHIVlacZ was 380% in the presence of 100 U/ml α-TNF. Correlation between effect of transactivation in these two distinct models may be attributed with the analogous levels of regulation of the genes expression such as NF-kB. Obtained results make possible to use Jurkat-tat cells transfected with plasmid pHIVlacZ as supplemental model for screening of HIV inhibitors or activators, allowing to evaluate the effect of α-TNF antiviral effect of test compounds without dangerous work with infectious virus.

56

HIV-1 PREINTEGRATION COMPLEX IS TRANSPORTED TO THE NUCLEI ALONG THE CELLULAR MEMBRANES.

A.Bukrinskaya.I.Alymova,O.Bogdan,G.Vorkunova,Yu.Tentsov.D.I.Ivanovsky Institute of Virology,Moscow,Russia.

HIV-1 preintegration complex (PC) contains inner proteins including matrix protein (MA) but not p24.MA possesses nucleophylic signal and is involved in nuclear targeting of PC. To enter the nuclei,MA needs phosphorylation at tyrosine acquired in virus producing cells and at serine acquired in target cells. To determine which way is used to reach the nuclei,the infected cells were labeled with 3H-leucine,3H-myristic acid or 32P, the proteins were immunoprecipitated from subcellular fractions and analyzed by PAGE followed by autoradiography and by Western blot.p24 was found in cytosol while MA was detected in nuclei and in membrane fractions 30 min - 8 h after infection.When infected cells were treated with anti-HIV membranotropic drugs such as dextran sulfate and adamantane derivarives, viral RNA and MA were not found in nuclei but were revealed in membrane fraction. We suggest that PC is transported to the nuclei along the membranes and membranotropic drugs are perspective in blocking intracellular transport of PC.

57

DETECTION OF UNINTEGRATED FORMS OF HIV-1 DNA IN CELL CULTURES AND IN CLINICAL SAMPLES

N.M. Gashnikova, O.A. Mamayeva, O.A. Plyasunova, A.G. Pokrovsky. State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russia.

Human immunodeficiency virus type 1 (HIV-1) DNA exists in different forms-integrated provirus and unintegrated proviral DNA, represented the linear double-stranded DNA and two circular forms that contain either one or two long terminal repeats (LTR). The content of proviral DNA was determined by two-step polymerase chain reaction (PCR) with nested primers. PCR assays were developed to detect specifically the 1-LTR and 2-LTR circular HIV-1 DNA. A set of reference primers for the region of env gene was used for detection of the total proviral DNA. Content of the circular and total forms of HIV-1 proviral DNA in various cell culture lines infected with HIV-1 reference strains and in the limphocytes isolated from HIV-infected patients at the different stages of HIV-infection was studied. This content was shown to vary. The correlation between accumulation of 2-LTR circular HIV-1 DNA and activation of HIV-infection was demonstrated. The search for peculiarities of individual viral DNA species accumulation will be useful for further studies of HIV infection pathogenesis.