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Evaluation of Antiretroviral Drugs Against Rauscher MuLV by ELISA

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An automated method has been developed for assaying antiviral drug activity against Rauscher murine leukemia virus (R-MuLV). The test system is based upon ELISA technology. Murine embryonic cells (SC-1) are plated in 96-well tissue culture plates (6 x 10^3 cells/well). After overnight incubation, the subconfluent monolayers are treated with DEAE-dextran. Following a PBS wash, appropriate concentrations of the test compounds are added to replicate wells(4). A standard dose of virus is then added to the wells. Controls include 6 wells containing virus without drug (virus controls) and 6 wells containing medium without drug or virus (negative or cell controls). In parallel, SC-1 cells in 96-well plates are exposed to medium (cell controls) and test compounds for cytotoxicity determination by an MTT assay. The plates are incubated for 4 days at 37° in a humidified 5% CO_2 atmosphere. Medium is removed from the antiviral plates, the cells washed once with PBS, and then fixed with cold ethanol:acetone (2:1). After drying, a blocking agent (2% fetal bovine serum in PBS) is added and the plates incubated. The blocking agent is removed and the cells exposed to goat anti-Rauscher p30 antibody. After incubation, the cells are washed 5 times with PBS and horseradish peroxidase labeled anti-goat antibody is added. The plates are incubated again and the excess antibody removed by 5 PBS washes. This is followed by substrate addition and incubation. The OD_{405} is read spectrophotometrically. The results, expressed as the % cytotoxicity and % virus inhibition, are presented graphically for ease of interpretation. The data can also be used to calculate therapeutic indices. Results obtained by this ELISA assay and the UV-XC plaque reduction assay will be compared. In addition, application of this ELISA method to drug combination evaluations will be presented. This work was supported, in part, by NCI Contract No. NO1-CM8-7274.

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The Inhibition Effects of Catechin Derivatives on The Activities of Human Immunodeficiency Virus Reverse Transcriptase and DNA Polymerases, P.Z. Tao, T. Zhang, P. Zhou, S.Q.Wang, S.J.Cheng and J.Y.Jiang, Institute of Medicinal Biotechnology, CAMS, Beijing, P.R.China

Catechin derivatives including (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC) and green tea extract (GTE) were found to inhibit the activities of cloned human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT), duck hepatitis B virus replication complexes reverse transcriptase (DHBV RCs RT) , herpes simplex virus type 1 DNA polymerase(HSV-1 DNAP) and cow thymus DNA polymerase α (CT DNAPα). It shows that EGCG and ECG both emerge as very potent inhibitors of HIV-1 RT. According to the IC50 values for HIV-1 RT these compounds can be orderd as EGCG 0.0066 uM/L > ECG 0.084 uM/L > GTE > 0.1 ug/ml > EGC 7.2 uM/L. DHBV RCs RT is the most insensitive enzyme. The kinetic study displays that EGCG shows a mixed inhibition with respect to external templet inducer poly(rA)·oligo(dT)12-18 and a noncompetitive inhibition with respect to substrate dTTP for HIV-1 RT. Bovine serum albumin significantly reduced the inhibitory effects of catechin analogues and GTE on HIV-1 RT. In tissue culture GTE showed inhibitory effect on cytopathic effect of coxsackie virus B3, but no inhibition on cytopathic effects of HSV-1, HSV-2, infouenza A and influenaa B viruses.