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Comparison of the Activity of 4-Amino and 4-Hydroxyamino Acyclic Tubercidin Analogs Against Cytomegaloviruses and Evaluation of Cytotoxicity in Mammalian Cell Lines. M.R. Nassiri, S.R. Turk, E.R. Kern, J.P. Robinson, M.J. Cameron, J.S. Pudlo, L.B. Townsend, and J.C. Drach. University of Michigan, Ann Arbor, MI, 48109; University of Alabama at Birmingham, Birmingham, AL 35294; and Purdue University, West Lafayette, IN 47907, U.S.A.

Two series of 5-halo-7-dihydroxypropoxymethyl pyrrolo[2,3-*d*]pyrimidine nucleosides with either NH_2 or NHOH in the 4-position were evaluated for activity against murine and human cytomegalovirus (HCMV) and for cytotoxicity. In both series, the 5-Br analogs were most active. In yield reduction experiments with HCMV both Br compounds reduced HCMV titers by $1x10^5$. The 4-NH_2 analog (compound 183) was more active than the 4-NHOH analog (compound 299) (IC_{90} =1.9 and 10 μM, respectively). In plaque reduction experiments with murine cytomegalovirus (MCMV), compound 183 also was more potent than compound 299 (IC_{50} = 0.016 and 0.035 μM, respectively). Both compounds inhibited the growth of uninfected cells but compound 183 did so at 1-10 μM whereas 10-100 μM compound 299 was required. Flow cytometric evaluation of cellular DNA content revealed inhibition by both compounds resulted from an accumulation of cells in early S phase and a reduction of cells in the G_2/M phase of the cell cycle. In plating efficiency experiments utilizing B-mix cells (an adenosine deaminase deficient cell line), a concentration of approximately 100 μM compound 299 was required for 50% inhibition in both wild type and adenosine kinase deficient cells. This suggests that adenosine kinase is not involved in the activation of compound 299. Both compounds were evaluated in a MCMV mouse model which is 90% lethal in untreated animals. At 3.1 to 5.6 mg/kg, compound 183 gave >90% survivors whereas compound 299 gave 60 - 80% survivors at 12.5 mg/kg. In a viral pathogenesis study, compound 183 reduced blood and tissue MCMV titers in a manner similar to that produced by ganciclovir. We conclude that the greater therapeutic efficiency of compound 183 is related to its greater potency *in vitro*. These studies were supported by N.I.A.I.D. contracts NO1-AI-42554, -62518, -72641 and -82518.

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ESTABLISHMENT OF AUTOMATED ASSAY SYSTEMS FOR DETECTION OF ANTI-HSV AGENTS

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New assay methods have been developed for the detection of anti-HSV agents using a microtiter plate. The purpose was to replace the classical plaque or focus reduction method by a more sensitive and reproducible test procedure which could allow us automated manipulation and calculation of the 50% inhibitory concentrations with computer. First, we examined whether the MTT (a tetrazolium dye) method, which had already been used for the detection of anti-HIV agents, could be applied to the anti-HSV assay. Monolayer cells such as Hela or human embryonic fibroblasts were not suitable for the MTT assay, since the amount of reduction of MTT by these cells did not correlate with the number of viable cells. We employed, therefore, a B-lymphoblastoid cell line NC-37 as the target line, which was found to be highly susceptible to HSV. After optimization of the assay conditions, the method proved as sensitive as the plaque reduction method. Next, we tried to evaluate several compounds for their inhibitory effect on HSV antigen expression. The method was based on an ELISA technique that could directly determine the amount of virus antigens in each well of a microtiter plate. Furthermore, it may also be applicable to the evaluation of compounds for their anti-VZV and anti-CMV activities.