34

Antiviral Activity of Anti-Cytomegalovirus Agents Assessed by a Flow Cytometric Method and DNA Hybridization Technique

R. Snoeck, D. Schols, G. Andrei, J. Neyts and E. De Clercq
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

Phosphonylmethoxyalkylpurine and pyrimidines, particularly (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) and (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) and their cyclic forms, are selective and potent inhibitors of human cytomegalovirus (CMV) replication *in vitro*. Their anti-CMV activity and that of the 3-deaza derivative of HPMPA and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, ganciclovir), have been monitored by a flow cytometry-based method and DNA hybridization. The cells were stained at 7 days post infection with a murine monoclonal antibody (kindly provided by S. Michelson, Institut Pasteur, Paris) directed against a late antigen protein. A fluorescein conjugated $F(ab')_2$ fragment was then added to visualize the positive cells. For the DNA hybridization assays the commercial kit (Hybriwix) containing CMV DNA probes labelled with I^{125} was used. The IC_{50} values obtained by the flow cytometric assay were : 0.1, 0.2, 0.2 and 0.25 µg/ml for HPMPC, HPMPA, 3-deaza-HPMPA and DHPG, respectively. These values are comparable to those obtained in a conventional virus plaque reduction assay. The DNA hybridization assays revealed that at 40, 10 and 1 µg/ml, all compounds completely blocked viral DNA synthesis. At 0.04 µg/ml, HPMPC, HPMPA and 3-deaza-HPMPA caused about 50% reduction in viral DNA synthesis, as compared to only 20% for DHPG. Thus, flow cytometry and DNA hybridization represent adequate methods to quantify the inhibitory effects of antiviral compounds on CMV replication.

35

A Rapid Assay for Cytomegalovirus Antiviral Sensitivity. T. Kino, E. Kern, W. Britt, R. Whitley, and F. Lakeman, Fugisawa Pharmaceuticals, Co., Ltd., Tsukuba, Tokodai, Japan and the University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

A rapid, specific enzyme-immunoassay was developed to determine the sensitivity of cytomegalovirus (CMV) isolates to selected antiviral drugs. Monoclonal antibodies (MAb), P63-27, directed against the major immediate-early viral protein, and, 28-21 which is specific for a late viral DNA-binding protein, were employed in a plaque enumeration assay. Bound Mab was detected using a biotin-avidin-HRP indicator. Following inoculation of 200 plaque forming units of the AD-169 strain of CMV, sequential dilutions of antiviral drugs were assessed to determine the ID_{50} and ID_{90} values. We tested Acyclovir (ACV), DHPG, 2'-O-phosphonylmethyl)-5-9-(2,3-dihydroxypropyl) adenine (-HPMPA), 1-[(3-hydroxy-2-phosphonyl methoxy) propyl] cytosine (HPMPC), phosphonoformic acid (PFA), castanospermine (CAST), human lymphoblastoid interferon (h-IFN-α-2), and human fibroblas interferon beta (h-IFN-β). The inhibitory effects of ACV, DHPG, HPMPA, HPMPC, and PFA were detected with the 28-21 MAb but not P63. The relative potencies of these drugs against CMV were: HPMPC > HPMPA > DHPG > ACV = PFA. In contrast, the antiviral activity of H-IFN was detected by using either P63 or the 28-21 MAb. CAST did not have antiviral activity in this assay.