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A Sensitive and Accurate Assay System for the Screening of Antiviral Compounds against Herpes Simplex Virus Type 1 and Type 2.

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A highly sensitive and accurate assay system was developed for *in vitro* evaluation of anti-herpes simplex virus (HSV) agents using MTT method with human embryonic lung fibroblast (MRC-5) cells. The assay system established in our laboratories was found to be highly sensitive to both HSV-1 and -2. Moreover, BVaraU and AraA, which are not able to evaluate antiviral efficacies in Vero cells (for BVaraU) or NC-37 cells (for AraA), were also proved their antiviral activities in this assay system. To establish the assay system using MTT method, we examined following conditions ; 1) cell line for assay, 2)virus titer for inoculation, 3) incubation times with virus, and 4) incubation times with MTT. As a result, confluent MRC-5 cells cultured in a flat-bottomed 96-well microtiter tray were infected with either HSV-1 KOS strain or HSV-2 G strain of 25 $TCID_{50}$ in the presence of various concentrations of test compounds. The cultures were incubated for 6-7 days at 37℃. Then the cultures were further incubated for 24 hours with MTT. The optical density (OD) of formazan was read in a computer-controlled microplate reader. The EC_{50} values of ACV obtained by this assay were 0.032 μg/ml for HSV-1 and 0.095 μg/ml for HSV-2, respectively. These EC_{50} values were equivalent to those of plaque reduction method. The EC_{50} values of several anti-HSV agents (BVDU, BVaraU, DHPG, AraA and others) were found to be similar to those obtained by the plaque reduction method. These results indicate that MTT assay with MRC-5 cells is useful for screening anti-HSV-1 and -2 agents.

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SUSCEPTIBILITY OF HCMV CLINICAL ISOLATES TO GANCICLOVIR (GCV) AND HPMPC.

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Fifty-two clinical isolates of human cytomegelovirus (HCMV) were examined for their susceptibility to GCV and HPMPC in a plaque reduction assay. These HCMV strains were isolated from bone marrow transplant recipients, patients with malignant tumor and those with CMV hepatitis. The mean EC_{50} values (the drug concentration required to reduce the number of plaque by 50%) of GCV and HPMPC for all HCMV isolates were 0.73 ± 0.2 μg/ml (range 0.48-1.85 μg/ml) and 0.15 ± 0.04 μg/ml (0.11-0.27 μg/ml), respectively. No resistant HCMV strain to both GCV and HPMPC was found in all the strains tested. However, one strain isolated from a patient without exposure to GCV showed intermediate susceptibility (EC_{50}: 1.85, EC_{90}: 5.9 μg./ml) to this drug. Twenty-three HCMV strains obtained from the clinical specimens, which were cultured in the presence of GCV, were randomly chosen for the drug susceptibility test: no resistant strains were isolated, however, six had intermediate susceptibility to GCV (EC_{50}: 1.6-2.25 μg/ml, EC_{90}: 4.3-7.4 μg/ml). Seven strains isolated in the presence of HPMPC were also examined for their susceptibility to HPMPC: none of them were found to be resistant or intermediate susceptible. Exposure to GCV for 1-3 months did not alter the susceptibility of clinical isolates.