

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 TCID₅₀ in the presence of various concentrations of test compounds. The cultures were incubated for 6-7 days at 37°C. 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 EC₅₀ 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 EC₅₀ values were equivalent to those of plaque reduction method. The EC₅₀ 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.

SUSCEPTIBILITY OF HCMV CLINICAL ISOLATES TO GANCICLOVIR (GCV) AND HPMPIC. Kenji Konno¹⁾, Tomoyuki Yokota¹⁾, Masanori Baba²⁾ and Shiro Shigeta²⁾. Rational Drug Design Laboratories¹⁾, Fukushima (960-12) and Department of Microbiology, Fukushima Medical College²⁾, Fukushima (960-12), Japan.

Fifty-two clinical isolates of human cytomegalovirus (HCMV) were examined for their susceptibility to GCV and HPMPIC 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 EC₅₀ values (the drug concentration required to reduce the number of plaque by 50%) of GCV and HPMPIC 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 HPMPIC was found in all the strains tested. However, one strain isolated from a patient without exposure to GCV showed intermediate susceptibility (EC₅₀: 1.85, EC₉₀: 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 (EC₅₀: 1.6-2.25 µg/ml, EC₉₀: 4.3-7.4 µg/ml). Seven strains isolated in the presence of HPMPIC were also examined for their susceptibility to HPMPIC: 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.