155

The Development of an In Vitro Antiviral Susceptibility Assay Using Alamar Blue. T.H. Belhorn, M.D., Ph.D., and S. Azar. Univ. of Texas Health Sciences Center, Houston, Texas, U.S.A.

Antiviral susceptibility assays have classically relied upon the quantitation of virus or viral products (DNA, protein) in the determination of drug efficacy, although alternate methodologies have been proposed and utilized. Alamar Blue is a fluorometric / colorimetric oxidation-reduction indicator which has been useful in cellular proliferation and cytotoxicity assays. We report the development of an assay incorporating the use of this indicator to monitor and quantitate viral replication in cell cultures. Alamar Blue absorbance data were used to distinguish not only HSV-infected from non-infected Vero cells, but the degree of viral replication in different cultures. In antiviral susceptibility assays, the degree of reduction of the indicator (absorbance) directly correlated with the decrease in virus yield (PFU/ml) seen with increasing concentrations of an effective antiviral agent. The correlation coefficient for the relationship between alamar Blue absorbance (OD) and PFU/ml ranged from 0.8863 to 0.9705 ($p < 0.05$ to < 0.006) in studies involving HSV-1 or HSV-2 at a multiplicity of infection of 1 to to 5 PFU/cell assayed 48 to 72 hours post infection. Drug resistance of virus isolates was easily demonstrated in the assay. Considering the simplicity, rapidity, and reliability of this assay, alamar Blue may provide a valuable system for screening virus isolates for resistance to antiviral agents.

156

Development of Anti-HSV Screening System Using Suspension Cell line and Screening Several Nucleoside Analogues in This Method T. Kira[1], A. Kakefuda[2], H. Awano[2], S. Shuto[2], A. Matsuda[2], M. Baba[3], M. Saneyoshi[4], S. Shigeta[1]
[1] Department of Microbiology, Fukushima Medical College, Fukushima 960-12, Fukushima [2] Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Hokkaido [3] Division of Human Retroviruses, Center for Chronic Viral Diseases, Faculty of Medicine, Kagoshima University, Kagoshima 890, Kagoshima [4] Department of Biological Sciences, The Nishi-Tokyo University, Uenohara 409-01, Yamanashi, Japan

We developed rapid and sensitive HSV screening system by using a suspension cell line RPMI8226. RPMI8226 cells were derived from human myeloma cells and proved to be sensitive for KOS (HSV-1 standard strain), A4D (HSV-1 ACV resistant strain), Hangai (HSV-1 clinical isolate) and G (HSV-2 standard strain) strains though RPMI8226 were not sensitive to clinical isolate of HSV-2. Several known anti-herpes compounds were examined in our system against KOS strain and G strain and ACV, BVaraU, AraA, DHPG, PFA and DS showed enough inhibition for both HSV proliferation. We will report about novel rapid and sensitive HSV screening system at first.

We examined many novel nucleoside analogues in our screening system and found some isonucleoside analogues and 2-thionucleoside analogues showed Anti-HSV-1 and -HSV-2 activities. Some of them were also inhibitory for A4D (ACV resistant Strain), though anti-HSV activity was lower than ACV.