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Antiviral Activity of 5'-O-Methylphosphonyladenosine.
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Investigating on antiviral activity of 5'-O-alkylphosphonylnucleosides, 5-O'-methylphosphonyladenosine (FCE 25148A) has been synthesized and showed an in vitro broad spectrum activity against RNA viruses. Besides some evaluation of in vivo activity was also performed. The in vitro tests were carried out generally on monolayers of human Hep # 2 cells with the exception of hamster BHK cells for Influenza virus and murine L 929 cells for Columbia SK virus. FCE 25148A exhibited activity against Respiratory Syncytial (Infectious Virus ID_{50} 20 μM), Semliki Forest (24 μM), Coxsackie (58 μM), Vaccinia (78 μM), Influenza (35 μM) and Columbia SK virus (60 μM). Cytotoxicity ($T.C.ID_{50}$) for the host cells was observed at concentration of 360 μM for Hep # 2 cells, ⩾ 400 μM for BHK cells and 270 μM for L 929 cells. In vivo tests were carried out in mice infected with Influenza virus by i.n. route, Semliki Forest and Vaccinia virus by i.c. route and Columbia SK by i.p. and i.c. routes. Therapeutic activity was observed against Influenza, Vaccinia and particularly against Semliki Forest virus. No in vivo activity was seen on encephalomyocarditis virus (Columbia SK) infection of mice.

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An Automated Evaluation of Antiviral Compounds *In Vitro* Against Representatives of Several RNA Virus Families. W. M. Shannon[1], J.J. Kirsi[1], T.P. Monath[2], and J.W. Huggins[2]. Southern Research Institute, Birmingham, AL 35255, USA, and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701, USA[2].

A rapid *in vitro* automated MTT (tetrazolium dye) assay protocol has been used in Vero-76 cells to evaluate prospective antiviral compounds against selected representatives of the following RNA virus families: Bunyaviridae (Punta Toro virus - Adames strain; sandfly fever virus - Sicilian strain), Togaviridae (Venezuelan equine encephalomyelitis virus - Trinidad donkey strain), Flaviviridae (yellow fever virus - Asibi strain; Japanese encephalitis virus- Nakayama strain). The daily, parallel assay results have been compared to results with the positive control compounds ribavirin and selenazofurin. The active compounds have been classified according to their maximum % CPE-inhibition values into four activity levels: (1) inactive: compounds with % CPE reduction of 0 - 24%; (2) moderate activity: compounds with % CPE reduction of 25 - 49%; (3) good activity: compounds with % CPE reduction of 50 - 94%; and (4) excellent activity: compound with % CPE reduction of 95 - 100%. Compounds within these activity levels have been further prioritized according to their Total Antiviral Index (TAI) = (area between cytotoxicity and antiviral curves) and Selectivity Index (SI = $TC25/IC_{50}$ values). This evaluation selects which compounds proceed to confirmation testing and then advance further into *in vivo* testing with appropriate animal models. Using the automated MTT assay, over 1000 compounds have been tested against each virus during the 6-month period for which data are presented. With this technique, several compounds have been found to be more potent antiviral agents *in vitro* than the positive control compounds. The automated MTT assay procedure provides a rapid and quantitative measurement of selective antiviral efficacy which yields data that compare well with the results of traditional microscopic CPE-inhibition assays and it has been found to be extremely useful in the primary *in vitro* screening of large numbers of submitted compounds. Supported in part by U.S. Army Medical Research Acquisition Activity Contract No. DAMD17-86-C-6013.