100

Mechanism Of Action Study Of Ribavirin On Surrogate Hepatitis C Animal Virus---Bovine Viral Diarrhea Virus

J Troxell, M Wenzel, R Buckheit Jr. and M Huang. Infectious Disease Research Department, Serquest, Southern Research Institute, Frederick, Maryland 21701-4756

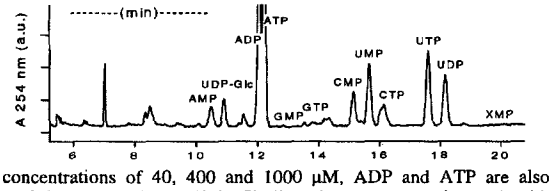
Ribavirin, a nucleotide analogue with a structure similar to that of azathioprine, is the only drug approved by FDA for HC treatment in addition to interferon-α2b. Unfortunately, the mechanisms of the action of ribavirin against HCV has not been defined.

In this study, we investigated mechanisms how ribavirin inhibits HCV replication using HCV surrogate animal virus, bovine viral diarrhea virus (BVDV), in vitro. Initially, we established a high throughput cytoprotection assay to evaluate the compound activity against BVDV. We found that ribavirin has a moderate activity against BVDV in our system with IC50 ~1.7ug/ml in average. In addition, we evaluate the combination effect (synergy, additivity, antagonism) of ribavirin and interferons in the system. To delineate the target(s) of the compound(s), we performed time of removal assay. With this assay, we can exclude the possibility that the compound blocks viral entry and we can demonstrate whether the compound inhibits at the early stage or late stage. Furthermore, we monitor the rate of nascent viral RNA synthesis by the real time quantitative PCR. In addition, we evaluate whether ribavirin inhibits HCV NS2/3 protease and HCV IRES using the assays developed in our institute. The detailed testing results will be presented.

101

Ribavirin (RBV) Represses Guanylate Nucleotide Pools but Elevates Pyrimidine Pools in Mouse Lymphoma ML1210 Cells as Measured by Capillary Electrophoresis (CE). H.C. Vo, K. O'Brien, B. Conway, D.D.Y. Chen, and S.L. Sacks. Viridae Clinical Sciences, Inc. and The University of British Columbia, Vancouver, B.C., Canada.

Ribavirin, a nucleoside analog used for treatment of hepatitis C, is a competitive inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH). RBV-phosphorylated forms could be partially responsible for its antiviral activity. A previous study suggested that RBV may not inhibit IMPDH at relevant concentrations. In order to determine the mechanisms of RBV actions and interactions with other antivirals, it is essential to determine the extent of RBV perturbation of cellular nucleotide pools. Accordingly, CE was used to quantify these perturbations in mouse lymphoma ML1210 cells treated with varying RBV (4, 40, 400, 1000 μM). After a 4h incubation, nucleotide pools ($5x10^6$ cells/sample) were extracted, freeze-dried and analyzed by CE. The capillary was rinsed for 1min with 1N NaOH, followed by a 3min rinse with the separation buffer; sample injection was done hydrodynamically for 30sec, followed by a 30min separation at 30 kV using a 75 μm i.d. capillary 57 cm total length. Nucleotides were detected at 254 nm using a photodiode array. RBV abolished GMP and XMP to below the limit of detection, and reduced AMP by 80%, but elevated pyrimidine pools increasing CMP, UMP/DP/TP by as much as 3-fold. Results (4μM; 4h) are shown below:



At concentrations of 40, 400 and 1000 μM, ADP and ATP are also reduced by as much as 50%. Similar changes were observed with incubations up to 72h. Results showed that RBV is a potent IMPDH inhibitor at physiologically-relevant concentrations.

102

Inhibition Of Measles Virus Replication By 5'-Nor Carbocyclic Nucleoside Analogs. D.L. Barnard[1], V. Stowell[1], K.L. Seley[2], V.R. Hegde[2], S.W. Schneller[2], and R.W. Sidwell[1]. [1]Inst. for Antiviral Research, Utah State Univ., Logan, UT, USA and [2]Auburn University, Auburn University, AL, USA.

Despite intense efforts to increase measles virus (MV) vaccine coverage, it still causes significant morbidity and mortality throughout the world. MV infection occasionally results in severe, chronic, lethal disease. In an effort to develop therapies to supplement immunization strategies, a number of 5'-nor carbocyclic nucleoside analogs were evaluated for anti-MV activity in CV-1 monkey kidney cells. Of those compounds tested, VRH-23, VRH-24, KS723, KS753, KS797, and KS796 were found to significantly inhibit MV, strain Chicago. The EC_{50} values ranged from <0.1 μg/ml to 3 μg/ml as determined by CPE reduction assay and confirmed by neutral red uptake. KS753 and VRH-24 had the most potent with EC_{50} values, <0.1 μg/ml. In addition, the activity of each compound was verified in a virus yield reduction assay, KS723 and KS796 being the most potent inhibitors ($EC_{90} \leq 0.1$ μg/ml). The compounds were also tested against other MV strains and similarly inhibited those strains except for a strain designated as Bil. No virucidal activity was detected at concentrations that inhibited viral replication. The compounds were generally not toxic at concentrations ≥100 μg/ml in actively growing and stationary phase cells. These results suggest that these compounds might be clinically useful anti-MV virus agents.

Supported by contract NO1-AI-85348, Virology Branch, NIAID, NIH and AI31718, Department of Health and Human Services.

103

Use of Cotton Rats for Preliminary Testing of Potential MeaslesVirus Antivirals. PR Wyde[1], E Guzman[1], E De Clercq[2], J Neyts[2], A Matsuda[3], K Chetty[1] and BE Gilbert.[1] [1]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA; [2]The Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium and [3]School of Pharmaceutical Sciences, Hokkaido Univ., Sapporo, Japan.

Monkeys are the only animals currently available to test chemotherapeutic or biologic agents identified in cell culture assays as being active against measles virus (MV). MV can replicate in lungs of cotton rats and thus these rodents may be useful for initial in vivo testing. To evaluate this possibility, ribavirin, EICAR and PAMPS (compounds that have been reported to inhibit MV), were tested in tissue culture and in cotton rats for antiviral activity. The EC_{50} values obtained for ribavirin EICAR and PAMPs in tissue culture were 47, 8, and 0.5 μg/ml, respectively. The selective indices of these compounds were 16 (ribavirin), 94 (EICAR) and >2,000 (PAMPS). All three compounds exhibited antiviral activity in cotton rats. Pulmonary MV replication was profoundly inhibited by PAMPS, but only if this compound was given in close proximity to the time of virus inoculation. The minimal effective dose (MED) of EICAR and ribavirin were 120 and 360 mg/kg/day, respectively. None of the compounds tested manifested any overt cytoxicity. The data obtained suggest that cotton rats may be useful for the initial evaluation of antivirals against MV. Testing of other compounds is currently in progress.