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Minimum High Dose Ribavirin Aerosol for the Treatment of Influenza in Mice and Respiratory Syncytial Virus in Cotton Rats. B.E. Gilbert, P.R. Wyde, M.W. Ambrose, H.L. Mover, S.Z. Wilson. Baylor College of Medicine, Houston, TX, USA.

Ribavirin aerosol administration has been shown to be effective in the treatment of respiratory syncytial virus (RSV) infections in infants and in influenza A and B virus infections in young adults. Long treatment schedules and potential for environmental contamination have stimulated the need for alternative dosing schedules. In previous animal studies, high dose ribavirin (HDR) (60 mg/mL; 2 hr, twice daily) administration was as effective as the standard (20 mg/mL, 12-18 hr daily) treatment and a recent clinical RSV infant trial indicated that HDR was well tolerated. Thus, we attempted to determine the **minimum** dosage of ribavirin aerosol necessary for effective treatment of influenza and RSV. In RSV infected cotton rats, as little as 30 min of HDR, 3 times daily, but not 15 min, reduced viral lung titers/gm of tissue (1.1 vs 0.6 $\log_{10}$ reduction, respectively). In influenza A virus infected mice, 15 min of HDR, 3 times daily was effective; however, the intervals between administrations were important. Treatment for 45 min, once daily was not as effective as divided doses. Calculations of ribavirin concentrations in respiratory secretions following 15 min treatment with HDR indicated drug levels dropped below the ED_{50} for RSV and influenza viruses after about 8 hr. From these experiments, the minimal daily dosage of ribavirin was estimated to be 8-15 mg/kg for the treatment of influenza and RSV infection; to calculate the duration of HDR needed for effective treatment, the minute volume of the animal or man must be considered. In mice, cotton rats, infants and adults, the **minimum** time required for HDR administration would be approximately 0.75, 1.5, 2 and 4 hr/day, respectively.

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Masked Nucleotides: A Strategy to Introduce Therapeutic Nucleoside 5'-phosphates into Cells. D. Farquhar, B. Nowak, S. Khan, and W. Plunkett. The University of Texas System M. D. Anderson Cancer Center, Houston, TX.

Resistance to therapeutic nucleosides may arise from the depletion or absence of primary activating kinases. To overcome this problem, we have synthesized a number of neutral bis(pivaloyloxymethyl) [$(piv)_2$] esters of antiviral nucleoside 5'-phosphates as potential prodrugs of the parent 5'-mononucleotides. It was anticipated that these compounds would penetrate into cells by passive diffusion, then revert to the free 5'-mononucleotides after sequential cleavage of the pivaloyloxymethyl groups by cellular carboxylate esterases. dTMP $(piv)_2$ was selected as a model compound to investigate this strategy. During a 1 h incubation of [^{3}H] dTMP $(piv)_2$ with CEM cells that lack thymidine kinase (CEM TK$^-$), intracellular [^{3}H] dTTP levels increased progressively and [^{3}H] dThd was incorporated into DNA; neither dTTP formation nor DNA tritium incorporation was observed after similar incubation of CEM TK$^-$ with [^{3}H] dThd. Consistent with these findings, dTMP $(piv)_2$ was equally growth inhibitory (IC_{50} = 5 uM) to CEM and CEM TK$^-$, whereas dThd was effective only against CEM (IC_{50} = 15 uM). In comparative metabolism studies of AZT and AZTMP $(piv)_2$ in CEM TK$^-$, only the masked nucleotide gave rise to intracellular AZTMP. These findings suggest that masked nucleotides may circumvent kinase deficiency and deliver therapeutic nucleotides into cells. (Supported by Grant AI-28213).