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Antiretroviral Activity of Ribonucleotide Reductase Inhibitors Hydroxyurea, Didox and Trimidox in the *in vivo* Rauscher Murine Leukemia Virus (RMuLV) Model. **P.L. BLACK[1], S. C. KUNDER[1], B. E. HALL[1], H.L. ELFORD[2], and M. A. USSERY[1]. [1]U.S.E.D.A., Rockville, MD; [2]Molecules for Health, Richmond, VA.**

Recently the ribonucleotide reductase (RR) inhibitors, specifically hydroxyurea, have gained attention for their *in vitro* activity against HIV. These compounds act upon cellular enzymes and would be less likely to produce resistant retroviral mutants than direct antiviral agents. We evaluated hydroxyurea and the other RR inhibitors 3,4-dihydroxybenzohydroxamic acid (Didox) and 3,4,5-trihydroxybenzamidoxime (Trimidox) in the RMuLV model. Splenomegaly, a marker for RMuLV infection, was reduced or completely inhibited by both Didox (500, 250, and 125 mg/kg, qd) and Trimidox (250, 125, and 62.5 mg/kg, qd) in a dose-dependent manner while hydroxyurea reduced splenomegaly to control levels at all doses (1000, 500, 300, and 100 mg/kg, qd). Preliminary evidence suggests that viremia was not reduced by these drugs, except by the lowest concentration of Trimidox, and at the highest level of hydroxyurea; this hydroxyurea concentration showed considerable toxicity. This discordance of two of the main markers of infection in RMuLV (splenomegaly and viremia) following treatment is unexpected and experiments directed at providing an explanation are ongoing. It may not be necessary for these compounds to inhibit virus replication as monotherapies since they have synergistic activity *in vitro* with nucleoside analogue antiviral drugs. Reduction of competing pools of naturally occurring deoxynucleotides by RR inhibitors probably accounts for this synergy. Further studies of the RR inhibitors, including combination studies with nucleoside analogues, are in progress.

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Phosphorylation of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) is greater in macrophages than in replicating lymphocytes and fibroblasts

E. Balestra, C.F. Perno, J. Balzarini*, S. Aquaro, G. Lazzarino, B. Tavazzi, D. Di Pierro, E. De Clercq*, R. Calio

Dept. Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Italy, and * Rega Institute for Medical Research, University of Leuven, Belgium

Dideoxynucleoside analogues (ddN) inhibitors of HIV-reverse transcriptase are usually more active in macrophages (M/M) compared to lymphocytes. The endogenous 2'-deoxynucleotide pools (dNTP) in M/M, lower than those in lymphocytes, determine an increased ratio ddNTP/dNTP, even if the intracellular triphosphorylation of ddN is limited. PMEA, a deoxyadenosine analogue with potent antiviral activity against HIV and DNA viruses, including herpes simplex virus (HSV-1), is the most potent nucleoside analogue tested in our M/M models. EC_{50} of PMEA in M/M infected by HIV-1 or HSV-1 is 0.009 to 0.02 uM respectively. These EC_{50} are about 400 fold lower than that found in fibroblastoid cells infected by HSV-1, and 200 fold lower than that achieved in lymphocytic cells infected by HIV-1. Even in the presence of cytokines enhancer of virus replication, such as macrophage-colony stimulating factor (M-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF), EC_{50} of PMEA in such M/M are in the range of 0.06-0.3 uM, still far lower than those found in fibroblasts and lymphocytic cells. To explain this potent activity of PMEA in M/M, we studied the intracellular phosphorylation of PMEA to its active form PMEA-diphosphate (PMEApp), and the levels of the endogenous counterpart dATP. dATP levels in M/M are 10 and 25 fold lower than those found in lymphocytes and fibroblasts respectively; the treatment with cytokines slightly increases the levels of dATP. More interestingly, and in contrast with other nucleoside analogues, the PMEApp levels in M/M, in the presence or absence of M-CSF or GM-CSF, are 6 and 10 fold greater in M/M than in lymphocytes and fibroblasts respectively. Thus, the ratio PMEApp/dATP is about 150 fold and 100 fold greater in M/M, and this explains, at least in part, the dramatic difference of the antiviral activity of PMEA in M/M with that found in other cells infected with HIV or HSV-1. The fine reasons of this enhanced phosphorylation of PMEA in M/M are still under investigation; nevertheless, overall results strongly support the clinical studies with PMEA derivatives currently ongoing in patients with diseases caused by retroviruses or DNA-viruses sensitive to the antiviral effect of PMEA.