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Inhibition of cytomegalovirus by metal chelators: investigations on metabolic pathways

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The antioxidant metal chelators desferrioxamine (DFO) and diethylenetriamine penta acetic acid (DTPA) proved to inhibit cytomegalovirus (CMV) replication *in vitro*. Since DFO acts intra- and extracellularly but DTPA only extracellularly the mechanisms of antiviral action might be different. Moreover, both chelators have different affinities to various metal ions. We wanted to find out in how far the anti CMV effects of DFO and DTPA are due to the inhibition of NF-κB, an important transcription factor for CMV which has been shown to be stimulated during infection. Therefore, NF-κB translocation experiments and gel shift assays were carried out to confirm this hypothesis. Nontoxic and *in vivo* attainable concentrations that were shown earlier to elicit significant anti-CMV activity proved to inhibit NF-κB *in vitro*. Further, the ability of the chelators to influence the constitutive or TNF-α-induced stimulation of the NF-κB dependent CMV IE1/2 enhancer/promoter in transfected (plasmid: pRR55) premonocytic HL-60 cells was studied. In five independent experiments DTPA did not influence the constitutive but significantly inhibited the TNF-α-stimulated pRR55 activity (mean: 45 %; n = 5). DFO neither influenced the constitutive nor the TNF-α-mediated activity of pRR55. These results suggest that the metal chelators DFO and DTPA elicit their anti CMV activity via different metabolic pathways.

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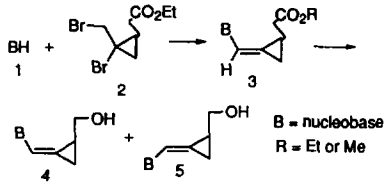
Synthesis and Isomeric Assignment of 2-Hydroxymethylcyclopropylidene-purines and -pyrimidines- New Antiviral Agents. Y.-L. Qiu and J. Zemlicka, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201; M. B. Ksebati, Central Instrumentation Facility, Wayne State University, Detroit, MI 48202; J. C. Drach, School of Dentistry, University of Michigan, Ann Arbor, MI 48019.

One-pot alkylation/elimination of nucleic acid bases or appropriate precursors 1 with E- and Z-dibromoesters 2 using K_2CO_3 in DMF at 100°C gave E, Z-esters 3 (1 : 2). Thymine (1, B = Thy) was alkylated as a bis-O-trimethylsilyl derivative. Reduction of 3 with diisobutyl-



aluminum hydride in THF afforded analogs 4 + 5. The latter isomeric mixtures were separated by crystallization (B = Cyt) or chromatography directly (B = 2-amino-6-chloropurine, Thy) or after derivatization (B = N^4-BzCyt and N^6-dimethylaminomethyleneadenine). Deprotection of the latter with NH_3 in MeOH gave Z- and E-isomers 4 and 5 (B = Cyt, Ade). Hydrolysis of 4 and 5 (B = 2-amino-6-chloropurine) with 80 % formic acid at 85°C afforded 4 and 5 (B = Gua) whereas reaction with NH_3 in MeOH furnished 4 and 5 (B = 2,6-diaminopurine). The isomeric assignment followed from NOE experiments. All Z-isomers 4 exhibit a downfield shift of H_8 (purines) or H_6 (pyrimidines) relative to E-isomers 5. Also, CH_2O protons in Z-isomers 4 (but not E-isomers 5) are magnetically non-equivalent. Supported by NIH grant CA32779.

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Selective Activity of 2-Hydroxymethylcyclopropylidene-methyl Purines Against Human Cytomegalovirus. J. C. Drach, B.Y. Fan, R.G. Ptak, J.M. Breitenbach, and K.Z. Borysko, University of Michigan, Ann Arbor, Michigan 48109; Y.-L. Qiu and J. Zemlicka, Karmanos Cancer Institute, Wayne State University, Detroit, Michigan 48201, U.S.A.

Antiviral evaluation of a series of 2-hydroxymethylcyclo-propylidenemethylpurines and pyrimidines revealed that the Z-isomers of the adenine (Z-Ade) and guanine analogs (Z-Gua) were particularly active against human cytomegalovirus (HCMV). Both compounds inhibited the Towne and AD169 strains of HCMV in plaque ($IC_{50} \cong 2$ μM) and yield reduction assays ($IC_{90} \cong$ 1-2 μM). Neither compound was visually cytotoxic at 100 μM in stationary HFF cells but Z-Ade was slightly more cytotoxic to growing KB cells than Z-Gua (IC_{50}'s ≅ 80 and >100 μM, respectively). HCMV resistant to ganciclovir (GCV) due to a mutation in UL97 (IC_{50}'s of wild-type and GCV^r virus = 2 and 8 μM for GCV, respectively) was slightly resistant to Z-Ade (IC_{50}'s = 1.6 and 3.8 μM, respectively) but not Z-Gua (IC_{50}'s = 2.4 and 1.6 μM, respectively). HCMV resistant to GCV due to a mutation in DNA polymerase was sensitive to both compounds. *In vitro* metabolism experiments showed that Z-Ade antagonized the phosphorylation of [^{3}H]GCV to a limited extent suggesting an overlapping pathway of phosphorylation. Time of addition studies revealed that Z-Gua acted before ribosyl-benzimidazoles (DNA processing inhibitors) in the viral replication cycle but at the same time as GCV thereby suggesting inhibition of viral DNA synthesis as the mode of action. This study was supported by grants U19-AI31718 and R01-CA32779 from N.I.H. and funds from the University of Michigan.

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Coupled *in vitro* Transcription/Translation as a Tool for the Study of Human Cytomegalovirus DNA Polymerase. T. Cihlar and J. M. Cherrington. Gilead Sciences, Foster City, CA 94404, USA.

The catalytic subunit (UL54) and accessory protein (UL44) of HCMV DNA polymerase (AD169 strain) have been cloned and expressed in an in vitro coupled transcription/translation reticulocyte lysate system. The influence of the 5'-nontranslated leader sequence on the efficiency of expression from the circular plasmids has been determined. For both UL54 and UL44 a truncated form of the alfalfa mosaic virus (AMV) RNA4 leader sequence was found to be superior over either the full-length AMV leader sequence or the original HCMV leader sequences of different lengths. DNA polymerase activity of the expressed UL54/UL44 complex was found to be dependent on salt concentration in the same manner as the activity of enzyme purified from HCMV-infected cells. Affinity of the in vitro expressed UL54 enzyme for the deoxynucleoside triphosphates is similar to that of HCMV polymerase purified from infected cells. Also its sensitivity to known inhibitors (cidofovir diphosphate, ganciclovir triphosphate and foscarnet) resembles purified HCMV polymerase and is not substantially influenced by the presence of UL44 protein. Three plasmids carrying single amino acid changes in UL54 which are known to confer in vitro reduced susceptibility of HCMV to both ganciclovir and cidofovir (L501I or K513N) or foscarnet (T700A) have been constructed and expressed. The relative specific activity of both the L501I and T700A mutant DNA polymerases is similar to wild type enzyme, whereas the K513N mutant retains less than 25% of the wild type activity. Further characterization of the mutant enzymes, including determination of their exonuclease activity and affinity for the inhibitors of HCMV replication, is now in progress.