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Benzimidazole Ribonucleosides: Mode of Action of TCRB in HCMV-Infected Cells. S.R. Turk, J.M. Black, K.Z. Borysko, C.A. Edwards, M.R. Nassiri, L.B. Townsend, and J.C. Drach. Departments of Biologic & Materials Sciences, School of Dentistry and Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, U.S.A.

Certain ribosyl benzimidazole nucleosides have been identified by us as possessing potent and preferential antiviral activity against human cytomegalovirus (HCMV). Mode of action studies with TCRB [2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole] have been initiated and the progress is reported herein. Time course studies using titer reduction assays to examine the inhibitory potency of TCRB when added at various times post-infection revealed that maximum antiviral effect was obtained if drug was introduced during the first 30 hr post-infection whereas diminishing effects were evident if drug was added at later times. These results indicate the compound is neither an inhibitor of virus attachment or penetration nor is it a contact inactivator of virion infectivity. Viral DNA synthesis was examined by CsCl isopycnic centrifugation and proved sensitive to inhibition by ganciclovir (GCV) but was not inhibited by 100 μM TCRB. HPLC analysis of ribonucleotide pools indicated a modest (0-33%) inhibition of the four rNTP pools present in HCMV-infected cells treated for five days with TCRB but revealed no new chromatographic peaks which could be attributed to phosphorylated metabolites of this compound. SDS-PAGE analysis of viral protein synthesis indicated a moderate (55-74%) inhibition by TCRB of the synthesis of the three major viral proteins found in infected cells. Electron microscopy indicated that significant numbers of nucleocapsids and dense bodies were synthesized in cultures treated with TCRB. A strain of HCMV resistant to TCRB was developed but was not cross-resistant of GCV. Similarly, a GCV-resistant clinical isolate was equally sensitive to TCRB as was its GCV-sensitive counterpart isolate. These data establish that TCRB and GCV are significantly different in their antiviral modes of action. This work was supported by contracts N01-AI42554, N01-AI72641 and grant U01-AI31718 from N.I.A.I.D.

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Structure-Activity Relationships of New Inhibitors of S-Adenosylmethionine Decarboxylase and Their Effect on the Replication of Human Cytomegalovirus. J. D. Rose, W. B. Forrister, Jr., T. H. Moss, III, E. L. White, G. Arnett, L.M. Rose, W. M. Shannon, and J.A. Secrist III. Southern Research Institute, Birmingham, AL USA.

Infection of human cells by human cytomegalovirus (HCMV) increases the intracellular level of polyamines, especially that of spermidine and spermine. We have previously reported on the synthesis and evaluation of a series of analogs of the substrate for S-adenosylmethionine decarboxylase (AdoMet-DC), an enzyme required for the synthesis of spermidine and spermine (Second International Conference on Antiviral Research, 1988). The series of inhibitors consists of analogs of compounds in which the reactive end of the methionine group of S-adenosylmethionine has been replaced by either a hydrazino, aminooxy, or hydrazinocarbonyl group. We have examined the effect of changing the length of the side chain for each series on the compounds' ability to inhibit AdoMet-DC and inhibit HCMV replication. The hydrazino and hydrazinocarbonyl series had the same optimal chain length for inhibition of the enzyme. In the hydrazino series the K_i for the ethyl compound was 0.008 ± 0.002 μM, for the propyl compound it was 0.002 ± 0.001 μM, and 0.008 ± 0.002 μM for the butyl compound. In the hydrazinocarbonyl series the K_i for the methyl compound was 92 μM, for the ethyl compound it was 0.240 ± 0.090 μM, 4.83 ± 0.84 μM for the propyl compound, and > 126 μM for the butyl compound. Statistically, there was no difference in the K_is for the ethyl, propyl, and butyl analogs in the aminooxy series (0.015 ± 0.003 μM, 0.021 ± 0.007 μM, and 0.011 ± 0.003 μM). Data on the effect of other modifications of the inhibitors in both the ring structure and side chain will also be reported. The relationship between the ability of these compounds to inhibit the enzyme *in vitro* and their ability to inhibit HCMV replication in cell culture has been examined. **Partially supported by NIAID Contracts N01-AI-42555 & N01-AI-72642.**