47

Metabolism of carbovir in CEM cells. William B. Parker, Bonnie J. Bowdon, Lucy M. Rose, Sue C. Shaddix, R. Vince, W.M. Shannon, and L. Lee Bennett, Jr., Southern Research Institute, Birmingham, Al 35205.

Carbovir (the carbocyclic analog of 2′,3′-dideoxy-2′,3′-didehydro-guanosine, CBV) is a potent inhibitor of human immunodeficiency virus type I (HIV) replication in vitro due to the inhibition of HIV reverse transcriptase by its 5′-triphosphate (CBV-TP). In this work we have evaluated the metabolism of carbovir and its interaction with 3′-deoxy-3′-azidothymidine (AZT) in CEM cells grown in culture. Carbovir was poorly metabolized to CBV-TP. Only 1 pmole per 10^6 cells was detected in cells treated with 100 μM CBV for 6 hours. A linear correlation was observed between the accumulation of CBV-TP and the extracellular concentration of CBV from 0.1 to 100 μM. Significant production of [^{3}H]GTP was observed in cells treated with [^{3}H]CBV. However, when CEM cells were treated with [^{3}H]CBV purified by HPLC, the incorporation of label into GTP was decreased 93%, indicating that the incorporation of label into GTP was due to a contaminant. Only 0.009 pmoles of CBV per 10^6 cells of CBV were detected in the DNA of CEM cells treated with 10 μM CBV, supporting our previous work with isolated DNA polymerases showing that CBV-TP was a very poor substrate for the host DNA polymerases. CBV and AZT have been shown to act synergistically against HIV replication. In an effort to explain this observation, we have compared the effect of CBV on the metabolism of AZT and visa versa. Neither compound had any affect on the metabolism of the other compound. Furthermore, neither CBV nor AZT affected the intracellular concentration of the deoxynucleoside triphosphates. Therefore, there is still no explanation for the synergism observed with these two agents against HIV replication. This work was supported by NIAID grant number AI29157.

48

In vitro selection of human immunodeficiency virus type 1 resistant to 3'-Azido-3'deoxythymidine.
Dianzani F., Antonelli G., Turriziani O., Riva E., Guanmu D.
Institute of Virology, University "La Sapienza", Rome Italy.

Zidovudine (AZT) resistant HIV-1 strains were obtained by propagation in the presence of inhibitory concentrations of AZT. A number of these variants are capable of producing infectious progeny in the presence of AZT concentrations (0.5-0.05uM) that completely inhibit the replication of H9 derived HTLV-IIIB, considered as the parental virus. These variants appared to be also less sensitive to other nucleoside analogs such as ddI and ddC. The characterization of one of these variants (HIV-11H) revealed that viral DNA measured at 5hrs p.i. by quantitative PCR is produced even in the presence of concentrations of AZT capable to completely suppress the production of HTLV-IIIB viral DNA. The same results were also obtained when HIV-11H was used to infect PBMC instead of lymphoblastoid cells. In fact, although with a slower replicaton in PBMC, this virus shows resistance to AZT when viral DNA is measured at 18-24hrs p.i, thus suggesting that a mechanism of escape at level of primary transcription does exist. However reverse transcriptase (RT) derived from HIV-11H, failed to show resistance to AZT 5' triphosphate in vitro. Preliminary observations seem to indicate that , surprisingly, variants less sensitive to AZT can be obtained also by growing HTLV-IIIB in the absence of AZT and this feature seems to correlate to replicative characteristics of such variants. Whether these findings may have pathogenetic significance and whether repeated replication may effect AZT resistance in vivo is being studied. Work supported by a grant from Ministero della Sanità (Progetto A.I.D.S.).