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Inhibition of HSV-1 DNA polymerase by (*R*)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine and (*S*)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine. M.E. Hagen, C.W. Cianci, B.J. Terry. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, N.J. 08543 U.S.A..

(*R*)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine,[(*R*)-BHCG] and (*S*)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine,[(*S*)-BHCG] triphosphates were synthesized enzymatically and purified by HPLC to identify which enantiomer of (*R,S*)-BHCG-TP (SQ 33,054-TP) is a polymerase inhibitor. (*R*)-BHCG-TP is the more potent and selective inhibitor of herpes simplex virus type-1 DNA polymerase. However, (*R*)-BHCG is a much poorer substrate than (*S*)-BHCG for HSV-1 thymidine kinase; (*R*)-BHCG is phosphorylated 25-fold slower than (*S*)-BHCG. (*R*)-BHCG-TP is a selective inhibitor of HSV-1 DNA polymerase, with an inhibition constant of 0.017 μM; (*R*)-BHCG-TP inhibits DNA polymerase α 20-fold less. Both (*R*)-BHCG-TP and (*R,S*)-BHCG-TP inhibit HIV reverse transcriptase using a poly rC:oligo dG template. (*S*)-BHCG-TP does not inhibit HSV-1 DNA polymerase or HeLa DNA polymerase α (K_i > 300 μM for each); nor does (*S*)-BHCG-TP inhibit HIV reverse transcriptase. Incorporation of (*R*)-[8-^{3}H]BHCG-TP and (*S*)-[8-^{3}H]BHCG-TP into a well-defined DNA template was investigated and compared to incorporation of [^{3}H]ACV-TP, [^{3}H]ganciclo-vir-TP and the natural substrate [^{3}H]dGTP. (*R*)-BHCG-TP, ACV-TP, and GCV-TP were incorporated by HSV-1 DNA polymerase, while (*S*)-BHCG-TP was not. Extension of the tritiated primer:template was carried out in the presence of [α-^{32}P]dCTP, dTTP, and dATP; following (*R*)-BHCG-TP incorporation, 10% of the DNA molecules could be elongated compared to dGTP extended primer:template. Similar results were observed for (*R,S*)-BHCG-TP and GCV-TP, whereas ACV-TP appears to be a chain terminator. From these results, we conclude that the DNA polymerase inhibitory activity of (*R,S*)-BHCG-TP (SQ 33,054-TP) was due to one enantiomer, (*R*)-BHCG-TP.

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Penciclovir: Mode of action studies in HSV-1, HSV-2, and VZV infected MRC-5 cells.
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Penciclovir has been shown to have potent activity against HSV-1, HSV-2 and VZV, yet has low toxicity to uninfected cells. Penciclovir, and its oral prodrug famciclovir, are currently in clinical trials. When uninfected MRC-5 cells were treated with penciclovir, low or undetectable concentrations of penciclovir-triphosphate were formed in the cells. However in HSV-1 infected cells, high levels of the triphosphate ester were produced. We have investigated the phosphorylation of penciclovir in HSV and VZV infected cells. The rates of phosphorylation of 10 μM penciclovir in HSV-1 and HSV-2 infected cells were comparable (1360 and 1200 pmol/(min . g of cells) respectively). Whereas penciclovir was phosphorylated at about the same rate throughout the 4 hour incubation, acyclovir was phosphorylated mainly in the first one or two hours, the initial/subsequent rates being ca. 40/0 and 145/12 pmol/(min g of cells) in HSV-1 and HSV-2 infected cells respectively. For penciclovir in VZV infected cells, the corresponding value was 140, but no phosphate esters of acyclovir were detected. Although a much higher concentration of the triphosphate of penciclovir than of acyclovir was formed in herpes infected cells, the higher level of triphosphate ester of penciclovir is required to inhibit the herpesvirus DNA polymerases. The Ki values of penciclovir triphosphate were 8.5 and 5.8 μM for HSV-1 and HSV-2 respectively and the corresponding values for acyclovir triphosphate were 0.07 and 0.07 μM. This provides an explanation for the comparable activities of penciclovir and acyclovir in standard cell culture antiherpes assays. However, following the removal of acyclonucleoside from the culture medium, the intracellular triphosphate ester of penciclovir was much more stable than that of acyclovir. The half-lives were 10 and 0.7 h respectively in HSV-1 infected cells and 20 and 1 h respectively in HSV-2 infected cells. Also, penciclovir triphosphate had a long half-life (8 h) in VZV infected cells. The efficient entrapment of the triphosphate of penciclovir within the herpes infected cells provides an explanation for the activity of penciclovir in cell cultures following short treatment times.whereas acyclovir is shown to have little activity.