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Chemical Stability and Intracellular Metabolism of 1-(*S*)-[3-Hydroxy-2-(Phosphonomethoxy)Propyl]-5-Azacytosine

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1-(*S*)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine [(*S*)-HPMP-5-azaC], the 5-azacytosine analogue of cidofovir, represents a new acyclic nucleoside phosphonate with pronounced activity against DNA viruses. In this study, we investigated (*S*)-HPMP-5-azaC for its chemical stability in solution and metabolism in cell culture. 5-Aza-cytosine, being a triazine, is prone to chemical degradation. We observed that, at neutral pH, (*S*)-HPMP-5-azaC undergoes spontaneous and reversible opening of the triazine ring structure, which is followed by slow decomposition to the deformylated and antivirally inactive degradation product. About 30% of the (*S*)-HPMP-5-azaC concentration remains intact after 6 months storage in cell culture medium at pH 7.4 and 4 °C. Upon HPLC analysis of methanol extracts from human lung carcinoma A549 cells incubated with 10 μM 6-^{3}H-[(*S*)-HPMP-5-azaC] during 72 h, as much as eight peaks were observed, with the predominant peak (∼40% of the total radioactivity) representing the presumed active metabolite (*S*)-HPMP-5-azaC-diphosphate. Further identification of the remaining peaks (presumably representing the (*S*)-HPMP-5-azaC-monophosphate-choline metabolite, and the deaminated metabolite (*S*)-HPMP-5-azaU and its corresponding phosphorylated forms) is underway. Thus, unlike cidofovir, (*S*)-HPMP-5-azaC appears to be relatively sensitive to enzymatic deamination. On the other hand, our striking observation that the radioactivity in the methanol-insoluble fraction from (*S*)-HPMP-5-azaC-treated cells is about ten-fold higher compared to cells receiving cidofovir, may suggest a more efficient incorporation of (*S*)-HPMP-5-azaC into DNA. Our ongoing experiments are aimed at comparing the formation and intracellular half-life of (*S*)-HPMP-5-azaC and its metabolites in several cell types, including primary human renal tubular cells.

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Influence of Substitutes in the Central Phenyl Ring of Capsid Function Inhibitors on Anti-coxsackievirus B3 Activity

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Coxsackievirus B3 (CVB3)-induced diseases can be medicated only symptomatically until now. Promising compounds to treat CVB3 infection are capsid function inhibitors. After binding into a small hydrophobic pocket of the viral capsid protein 1, they hinder viral adsorption and/or uncoating. Single amino acid substitutions within the hydrophobic pocket can abolish the antiviral activity. Whereas Ile1092 mediates susceptibility, Leu1092 and Met1092 induce resistance. During this study the relationship between substitutions of the central phenyl ring of capsid blockers and their antiviral activity against different CVB3 was examined. Derivatives of [(biphenyloxy)propyl]isoxazoles as well as pleconaril were synthesized. After determining cytotoxicity, antiviral tests were performed with CVB3 Nancy and CVB3 97927 consisting of Leu1092 and Ile1092, respectively. Additionally, the inhibitory activity against CVB1, CVB2, CVB4, CVB5, and CVB6 was determined.

Capsid function inhibitors with methyl in position 3 and 5 of the phenyl ring are not active against CVB3 Nancy but prevent CVB3 97927 multiplication. SAR studies revealed that compounds without any substitutes in the central ring inhibit multiplication of CVB3 Nancy but exhibited strongly decreased antiviral activity against CVB3 97927. After introduction of methyl or brome substitutes in position 3, the anti-CVB3 Nancy activity was sustained and the anti-CVB3 97927 activity restored. The derivative substituted with 3-OMe exhibits only low activity against CVB3 Nancy, but effectively inhibits the CVB3 97927-induced CPE. Surprisingly, derivative with two methyl groups in position 2 and 3 of the central ring is completely inactive. Results obtained with CVB1, CVB2, CVB4, CVB5, and CVB6 fully confirm the important role of substitutes at the central phenyl ring for antiviral activity.

In summary, the results of this study demonstrate the impact of position and size of substitutes in central ring of capsid blockers for their spectrum of antiviral activity. To achieve broad spectrum anti-enteroviral activity and prevent CVB3-resistance combinatory therapy seems to be necessary.

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