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Anabolism of 6-Methoxypurine Arabinoside in Varicella-Zoster Virus (VZV) Infected Human Fibroblasts. P. de Miranda*, K. K. Biron, T. C. Burnette, R. L. Miller, D. R. Averett, and T. A. Krenitsky. Burroughs Wellcome Co., Research Triangle Park, NC 27709, U.S.A.

The nucleoside analog 6-methoxypurine arabinoside (ara-M) is a potent and highly selective VZV inhibitor. After incubation with ^{3}H(G)ara-M, the differences in the uptake of radioactivity between the VZV (Oka strain)-infected and uninfected human fibroblasts were striking, with the VZV-infected cells accumulating up to 114-fold higher radioactivity. Ara-M was extensively anabolized in the infected cultures to mono-, di- and tri-phosphorylated nucleosides. The only phosphorylated form of ara-M detected was ara-M monophosphate, whose formation was highly VZV-TK dependent. The other major metabolites were ara-AMP, ara-ADP, and predominantly ara-ATP. After removal of ara-M from the medium, the intracellular half-life of ara-ATP was approximately 2.6 h. The anabolic pathway of ara-M leading to the formation of ara-ATP in VZV-infected cells was elucidated with the use of specific enzyme inhibitors. The adenosine deaminase inhibitor EHNA enhanced formation of ara-ATP. However, deoxycoformycin and coformycin, which inhibit both adenosine and adenylate deaminases, blocked ara-ATP formation, as did the adenylosuccinate synthetase inhibitor hadacidin. These results indicate that after the initial phosphorylation of ara-M by the VZV-coded thymidine kinase, ara-M monophosphate is demethoxylated by adenylate deaminase forming ara-IMP, which is converted to ara-ATP by the sequential action of cellular adenylosuccinate synthetase/lyase and nucleotide kinases. The involvement of adenylate deaminase in the anabolic pathway leading to ara-ATP formation was also supported by the ability of deoxycoformycin and coformycin, but not EHNA, to reduce the potency of ara-M as an inhibitor of VZV replication *in vitro*. A comparison of the intracellular accumulation and anabolism of radioactive ara-A with that of ara-M indicated much lower selectivity of ara-A and up to 8-fold lower levels of ara-ATP derived from ara-A than from ara-M.

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2'- and 5'-Ester Prodrugs of the Varicella-Zoster Antiviral Agent, 6-Methoxypurine Arabinoside

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Studies in the rat have indicated that 6-methoxypurine arabinoside (ara-M), a potent and selective varicella-zoster antiviral agent, undergoes extensive first-pass metabolism following oral administration. In an effort to improve the systemic availability of ara-M, a series of twenty-five 2'-esters and twenty 5'-esters of ara-M were synthesized and evaluated as prodrugs on the basis of urinary recovery of ara-M. In the 2'-ester series, the straight-chain aliphatics led to increased urinary recovery of ara-M, with the 2'-valerate, hexanoate, heptanoate, and octanoate esters being the most effective. 2'-Branched-chain aliphatic esters and 2'-aromatic esters generally did not increase the recovery of ara-M in the urine. In contrast, the 5'-aromatic esters and 5'-branched-chain aliphatic esters showed significant enhancements in urinary recovery of ara-M. A direct correlation between the amounts of ara-M excreted and the electron-donating properties of the substituents on the 5'-benzoate esters was observed. On the basis of additional studies on the solubility and stability of these prodrugs, the 2'-valerate ester of ara-M was selected for more extensive evaluation. Preliminary toxicological information indicating central and peripheral neurotoxicity in rats and monkeys was obtained. The studies to be presented will show that by proper choice of prodrug, the unfavorable pharmacokinetic properties of ara-M could be overcome.