

Comparison of the Phosphorylation of 4'-Ethyneyl 2',3'-Dihydro-3'-Deoxythymidine with That of Other Anti-Human Immunodeficiency Virus Thymidine Analogs[∇]

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Thymidine analogs, including 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxy-3'-deoxythymidine (D4T), are important antiretroviral agents. To exert antiretroviral activity, these analogs undergo a stepwise phosphorylation intracellularly to the active triphosphate metabolites. We previously reported that 4'-substituted D4T with an ethynyl group (i.e., 4'-ethynyl D4T) increased the anti-human immunodeficiency virus (HIV) activity and was active against multidrug-resistant HIV strains. 4'-Ethyneyl D4T is a better substrate for phosphorylation by human thymidine kinase 1 than D4T is. In this report, we first studied the enzymes involved in the phosphorylation of 4'-ethynyl D4T from monophosphate to triphosphate metabolites. The 4'-ethynyl D4TMP is phosphorylated by recombinant human TMP kinase with a K_m of $19 \pm 4 \mu\text{M}$ and a k_{cat} of $0.007 \pm 0.001 \text{ s}^{-1}$; the relative efficiency is about 9 and 15% of those of D4TMP and AZTMP, respectively. Several enzymes from crude cellular extracts, including nucleoside diphosphate kinase, pyruvate kinase, creatine kinase, and 3-phosphoglycerate kinase, could phosphorylate 4'-ethynyl D4T-diphosphate. The relative phosphorylation efficiencies of 4'-ethynyl D4TDP were about 3 to 25% of those of D4TDP and were generally similar to those of AZTDP. In T-lymphoid cell lines, there was a preponderant accumulation of 4'-ethynyl D4TMP, suggesting that TMP kinase could be the rate-limiting enzyme in the metabolism of 4'-ethynyl D4T. Although the same enzymes are involved in the stepwise phosphorylation of thymidine analogs, their behaviors in phosphorylating metabolites of 4'-ethynyl D4T are different from those of D4T and AZT. Qualitatively, the metabolism of 4'-ethynyl D4T is more similar to that of AZT than to that of its progenitor, D4T.