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(-)-5-Fluoro-2',3'-dideoxy-3'-thiacytidine , FTC, is Efficiently Anabolised to Nucleotides in Human Hepatocytes and CEM Lymphoblastoid Cells. D. J. Nelson, D. R. Averett, M. T. Paff, K. L. Prus, and L. W. Frick. Wellcome Research Laboratories, Research Triangle Park, NC 27709 USA.

FTC inhibits HIV-1 and hepatitis B virus replication in vitro more potently than its (+)-isomer. In Hep G_2 (2.2.15, subclone P5A) cells incubated with purified [6-^{3}H]FTC, 5'-mono-, di- and triphosphate (FTC-TP) were formed in a time- and concentration-dependent manner. In cells incubated with 0.01 uM FTC, the maximum level of FTC-TP, 0.11 pmol/10^6 cells, was reached in 3 - 6 hr. FTC was more efficiently phosphorylated to the 5'-triphosphate than its (+)-isomer, and levels were 3.6 and 0.2 pmol/10^6 cells, respectively, after 24 hr incubation with 1 uM of the respective compounds. In a washout experiment, the initial intracellular half-life of FTC-TP was 2.4 hr, which was followed by a lower, but persistent level of the triphosphate. FTC was converted to a putative diphosphocholine derivative, analogous to CDP-choline, whereas (+)-FTC was converted to both the diphosphocholine adduct as well as to the putative diphosphoethanolamine derivative, analogous to CDP-ethanolamine. FTC was not detectably deaminated at either the nucleoside or nucleotide level, whereas (+)-FTC was partially deaminated. Culture medium from the 24-hr incubation with 1 uM (+)-FTC contained approximately 0.2 uM (+)-5-fluoro-2',3'-dideoxy-3'-thiauridine ((+)-FTU), and (+)-FTU 5'-monophosphate was detected in these cell extracts. The corresponding (-)-isomer, FTU 5'-monophosphate, was not found in cell extracts from the experiments with FTC. Similar intracellular metabolic profiles were obtained in CEM lymphoblastoid cells incubated with FTC. The transmembrane influx of FTC was examined in uninfected Hep G_2 cells. The influx of FTC was only partially sensitive to inhibitors of nucleoside transport, indicating that its (-)-isomer may have multiple transport mechanisms. In contrast, (+)-FTC enters these cells by way of the NBMPR-sensitive, equilibrative nucleoside transporter. These metabolic results are consistent with the conclusion that FTC readily enters the target cell and that FTC 5'-triphosphate is responsible for the antiviral activities of FTC.

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Selection of Human Immunodeficiency Virus Type 1 (Hiv-1) Resistant to the Nucleoside Inhibitor 2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (Ftc) in Cell Culture due to a Single Mutation in the Reverse Transcriptase. M. Tisdale, N. R. Parry, S. D. Kemp and B. A. Larder, Department of Molecular Sciences, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K.

2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC) is a potent selective inhibitor of HIV replication, acting at the triphosphate level on the reverse transcriptase. The (-)-β enantiomer is about 20-fold more active than the (+)-β enantiomer*. In these studies, serial passage in MT4 cells of wildtype HXB-2D and the 3'-azido-3'-deoxythymidine (AZT) resistant infectious clone RTMC (67N,70R,215F,219Q) were made in increasing concentrations of the (-)-β enantiomer of FTC. One to two pass at 2-4 times the IC_{50} was sufficient to see a significant shift in sensitivity which, with further passage, increased to >1000-fold. Direct RT/PCR sequence analysis of the reverse transcriptase coding region obtained from virus infected cells at pass 6 (>60xIC_{50}) revealed a change at codon 184. When the mutation M184 to V was introduced into infectious clones of HXB-2D and RTMC, this change alone accounted for the reduction in sensitivity seen with FTC on passage. Both mutant viruses were also highly resistant to the (+)-β enantiomer of FTC and to 2'-3'-dideoxy-3'-thiacytidine (3TC). Serial passage in increasing concentrations of 3TC, with both HXB-2D and RTMC, selected resistant variants at a similar rate to FTC. No cross resistance was seen with AZT, dideoxyinosine or the non-nucleoside inhibitor nevirapine. Further, passage with FTC in combination with AZT, resulted in a slower rate of development of FTC resistant virus. These observations may have important implications for the possible use of FTC and 3TC in the future therapy of AIDS.

REFERENCE

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