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Membrane Permeation Characteristics of the Structurally Related Anti-HIV Agents 1592U89 and (-)-Carbovir in Human Erythrocytes and Human T-Lymphoblastoid CD4+ CEM Cells. T.P. Zimmerman, W.B. Mahony, B.A. Domin, and K.L. Prus. Wellcome Research Laboratories, Research Triangle Park, NC 27709, USA.

1592U89 ((1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol) is a carbocyclic guanosine analogue that is currently in Phase I clinical trials for treatment of human immunodeficiency virus infection. Structurally, 1592U89 and (-)-carbovir (CBV) differ only at the 6-position of the nucleobase: the 6-cyclopropylamino group of 1592U89 contrasts with the 6-oxy moiety of CBV. We compared membrane permeation characteristics of these two compounds at 20°C both in human erythrocytes and in human T-lymphoblastoid CD4+ CEM cells. Initial rates of influx were measured using a "papaverine-stop" assay. In human erythrocytes, 1592U89 influx was fast, nonsaturable (rate constant=220 pmol/s/mM/µl cell water), and not inhibited by nucleosides or nucleobases; CBV influx was slow, saturable (V_{max}=0.67 pmol/s/µl cell water; K_m=60 µM), and strongly inhibited by adenine or hypoxanthine. Similar qualitative results were seen in CD4+ CEM cells. However, in these latter cells, CBV influx rates (V_{max}=2.3 pmol/s/µl cell water; K_m=75 µM) were faster, and 1592U89 influx rates (rate constant=28 pmol/s/mM/µl cell water) were slower, compared to the corresponding rates in erythrocytes. Equilibrium studies further revealed that these compounds were concentrated intracellularly in both cell types but not in erythrocyte ghosts, suggesting cytosolic protein binding of 1592U89 and CBV following membrane permeation. We conclude that, in both cell types, the influx of CBV is relatively slow and primarily dependent on the nucleobase carrier, whereas the influx of 1592U89, a more lipophilic compound, occurs relatively rapidly by nonfacilitated diffusion.

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Distribution to the brain, protein binding and physico-chemical properties of 5-substituted 3'-fluoro-uridine derivatives, studied by microdialysis

N Borg, X-X Zhou*, L Ståhle
Clinical Pharmacology, Karolinska Institute, Huddinge Hospital, S-14186 Huddinge.
*Medivir AB, Lunastigen 7, S-14144, Huddinge, Sweden.

The aim of this study was to correlate physicochemical parameters of a series of 5-substituted 3'-fluorouridine derivatives (FUD) to plasma protein binding, half-life and distribution over the blood-brain barrier in the rat. The lipophilicity was determined as the partition coefficient in octanol-water. Microdialysis was used to study protein binding in human plasma (*in vitro*) and to sample (*in vivo*) the extracellular space of rats with microdialysis probes implanted into the striatum of the brain and the gastrocnemic muscle, muscle levels reflect free plasma levels. The compounds were analysed by high-performance liquid chromatography with UV-detection. The partition coefficients of the FUD's varied from 0.22 to 0.84 while the partition coefficient of thymidine was 0.08. The protein binding of the FUD's was around 80%. After s.c. administration (25 or 50 mg/kg) the brain and muscle extracellular levels differed substantially. Brain levels were 1/2 to 1/7 of muscle concentrations. Thus, the FUD's are able to penetrate the blood-brain barrier in the rat to a significant extent. A multivariate analysis demonstrated a relation between the physicochemical and the pharmacokinetic properties of uridine analogues. Half-life and protein binding increased with decreasing pKa while penetration to the brain was related to the partition into the octanol phase.