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## MODE OF UPTAKE, ACCUMULATION AND INTRACELLULAR DISPOSITION OF ZIDOVUDINE IN CULTURED 3T3-F442A PREADIPOCYTES

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**AIM:** Zidovudine (ZDV) accumulates and undergoes intracellular phosphorylation in murine 3T3-L1 cells. Here, using 3T3-F442A preadipocytes, we further characterized the mode of uptake, accumulation, intracellular disposition and efflux of ZDV.

**METHODS:** At varying time points we measured the initial rate of intracellular uptake of radiolabelled ZDV. Using standard *in vitro* and established HPLC methodologies, we sought to investigate the effects of specific inhibitors on the uptake/accumulation, intracellular disposition, and efflux of ZDV and its metabolites from preadipocytes. P-gp was identified in the cells using western blot technology.

**RESULTS:** ZDV uptake and intracellular accumulation was rapid, reaching equilibrium within 20 min. At equilibrium, nigericin (K<sup>+</sup>/H<sup>+</sup> exchanger) increased ZDV accumulation by 1.9-fold. The accumulation of ZDV and conversion to zidovudine triphosphate (ZDVTP) was pH-dependent, being maximal at pH 7.4 and least at pH 5.1. Monensin (Na<sup>+</sup> ionophore), brefeldin A (V-Type ATPase inhibitor), bafilomycin A1 and concanamycin A (both inhibitors of vacuolar type H<sup>+</sup>-ATPase and organellar acidification, respectively) increased the intracellular accumulation of ZDV. On the contrary, the uptake of ZDV was inhibited by dipyridamole (a nucleoside transport inhibitor) and the weak base NH<sub>4</sub>Cl. Inhibitors of P-gp (GF 120918 and verapamil) and MRP-selective inhibitors (MK 571 and probenecid) also increased the accumulation of ZDV. Similarly, some HIV protease inhibitors, but not all (nelfinavir, saquinavir, ritonavir, amprenavir, lopinavir and indinavir) enhanced the accumulation of ZDV. Furthermore, we demonstrate that in preadipocytes P-gp is involved in the efflux of ZDV and its

phosphorylated metabolites at rates of 0.61 fM/h, 5.19 fM/h, 0.83 fM/h and 0.17 fM/h for ZDV, zidovudine monophosphate (ZDVMP), zidovudine diphosphate (ZDVDP) and ZDVTP, respectively.

**CONCLUSIONS:** The uptake, accumulation and efflux of ZDV in 3T3-F442A cells were energy-dependent and pH-sensitive. P-gp is one of the efflux transporters that significantly reduced the intracellular accumulation of ZDV and its metabolites. While the overexpression of multidrug transporters on adipocytes may potentially reduce the accumulation of antiretrovirals in adipocytes, thus minimizing toxicity (such as lipodystrophy), it could accelerate the acquisition of viral resistance. Indeed, targeted inhibition of P-gp may overcome this problem.

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