In Vitro Evaluation of Novel Equilibrative Nucleoside Transporter (ENT) Inhibitors that Reduce Mitochondrial Toxicity of Nucleoside Analog Antiretroviral Drugs

Category: 1. Basic Science

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Aims and Background: Equilibrative nucleoside transporters (ENTs) facilitate transport of nucleoside substrates across the membrane. ENTs are expressed by mitochondria and contribute to nucleoside analog antiretroviral drug (e.g., nucleoside reverse transcriptase inhibitors, NRTI) transport, which can facilitate intra-mitochondrial accumulation and drug toxicity. Our goal was to evaluate our library of novel ENT inhibitor prodrugs to prevent mitochondrial (mt) toxicity.

Methods: Initial evaluation of the library assessed in vitro safety profile for twelve novel ENT blocker-prodrugs (dipyridamole esters, JKB-25 to 36) in human hepatoma cells (HepG2) for mt toxicity. Subsequently, efficacy studies were conducted with one selected non-toxic prodrug, JKB25, in combination with NRTI controls to potentially inhibit mt toxicity. Control compounds included lamivudine (3TC), 1-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl) thymine (FMAU), or zalcitabine [2',3'-dideoxycytidine; ddC]. The mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) were amplified in parallel by real-time PCR, and the amount of target mtDNA was normalized to the amount of an endogenous control relative to the untreated control.

Results: After 14 days in culture, 75% (9/12) of the JKB-ENT blocker-compounds in HepG2 cells were found to have no mtDNA or nDNA toxicity up to 100 µM. In combination studies with ratios of 1:1 and 1:10, JKB-25 prevented toxicity (mtDNA, IC₅₀ > 10 µM) compared to FMAU alone (IC₅₀ = 6.8 µM).

Conclusion: The non-toxic ENT inhibitor prodrug, JKB-25, had the ability to block mtDNA toxicity of FMAU in HepG2 cells and warrants further preclinical evaluation.

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