Abacavir and Didanosine Enhance Susceptibility to Acetaminophen-Induced Hepatotoxicity

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BACKGROUND
Liver disease is a leading cause of mortality in HIV-infected patients treated with a multidrug therapy known as combined Antiretroviral Therapy (cART). Although many factors are associated with these complications, significant antiretroviral drug-induced hepatotoxicity has been identified in up to 23% of HAART patients. Nucleoside/nucleotide analogue reverse transcriptase inhibitors (NRTIs) are an essential component of cART, and the liver toxicity they produce is usually associated with disrupted mitochondrial DNA replication. However, mitochondrial dysfunction is also produced by other mechanisms unrelated to inhibition of Pol-γ. We have analysed the acute effects of clinically relevant concentrations of the most widely used NRTIs on mitochondrial function and their impact on the viability of hepatic cells. To explore a possible synergism with other drugs whose hepatotoxicity is attributed to the acute undermining of mitochondrial function, we have also evaluated the effects of NRTIs in combination with acetaminophen (APAP).

AIM
To evaluate the acute effects of clinically-relevant concentrations of the most widely used NRTI, alone or in combination with acetaminophen, on mitochondrial function and cellular viability in hepatic cells.

METHODOLOGY
Hep3B cells were placed in gas-tight chambers and O2 consumption was measured with a Clark-type O2 electrode after addition of the pyrimidine analogues Lamivudine, Zidovudine and Emtricitabine, the purine analogues Abacavir (ABC) and Didanosine (ddI), or the nucleotide analogue Tenofovir. Following incubation with ABC or ddI (1h) intracellular ATP was measured by fluorescence (ATP Bioluminescence Assay Kit HSII, Roche), and mitochondrial membrane potential (Δψm) and reactive oxygen species production (both parameters of mitochondrial function) were analyzed by static cytometry with a fluorescence microscope (Olympus IX81). Cellular proliferation and cell cycle and viability were assessed by fluorescence microscopy and MTT assay, respectively, after 24 or 48h incubation. Further experiments were performed in the presence of different concentrations of acetaminophen (APAP). Data (n≥2) were reported as mean+/−SEM, and statistical significance versus vehicle was analyzed by one-way ANOVA.*p<0.05, **p<0.01, ***p<0.001 (vs control).

RESULTS

Fig.1: Effects of NRTI on O2 consumption. Addition of NRTI to Hep3B cells produced different effects according to the type of drug involved. Only ABC and ddI inhibited mitochondrial respiration at clinically relevant concentrations (indicated by an arrow).

Fig.2: Effects of ABC and ddI on mitochondria. 1-hour incubation with ABC and ddI produced a significant and concentration-dependent increase of ROS, and a decrease of mitochondrial membrane potential and intracellular ATP levels (Fig. 2A-2C). Levels of TMRM fluorescence after 24-hours treatment did not differ significantly between the vehicle and these drugs (Fig. 2D).

Fig.3: Mitochondrial and cellular toxicity of APAP. APAP exerted a cytotoxic effect in Hep3B at high concentrations of the drug (2.5-10 mM), while 1.25 mM induced low levels of toxicity, decreasing cell proliferation but not compromising cellular viability or mitochondrial function.

Fig.4: Analysis of mitochondrial function and cellular viability after co-administration of APAP. Co-administration of both stimuli (APAP and either ABC or ddI) enhanced the individual effects of each drug on mitochondrial function, cell count and viability.

CONCLUSIONS
Purine analogues produce an acute and slight inhibition of mitochondrial function, an effect that is counteracted by cellular defense mechanisms. A combination of ABC or ddI with low concentrations of APAP significantly increases the risk of APAP-mediated liver injury. Our findings are of considerable relevance given that APAP is currently prescribed to some patients taking NRTI.