Abacavir and Didanosine Enhance Susceptibility to Acetaminophen-Induced Hepatotoxicity

Ana Blas-García, Víctor M. Víctor, Alberto Martí-Rodrigo, Lara Milián-Medina, Nadezda Apostolova, Juan V. Esplugues

[1] Departamento de Farmacología, Facultad de Medicina, Universidad de Valencia, [2] FISABIO, [3] CIBERehd, Valencia, Spain.

CONTACT: ana.blas@uv.es; www.pharmacologyvalencia.com

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).

REFERENCES: Joshi D. et al. Lancet 2011; 277:1198-1209. Venhoff N. et al. Antivir Ther 2007; 12:1075-1085. Blas-García A. et al. Hepatology 2010; 52:115-125.Lederman J.C. et al. Am J Gastroenterol 2001; 96(12):3474-3475. Moling O. et al. Clinical Therapeutics 2006; 28(5):755-760.

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 O_2 consumption was measured with a Clark-type O_2 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 Bioluminiscence Assay Kit HSII, Roche), and mitochondrial membrane potential ($\Delta\psi m$) 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≥5) 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

Purine analogues produce an acute and slight inhibition of mitochondrial function, an effect that was counteracted by cellular defense mechanisms.

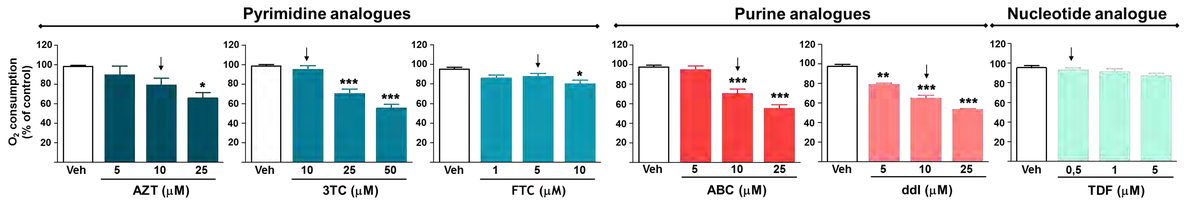
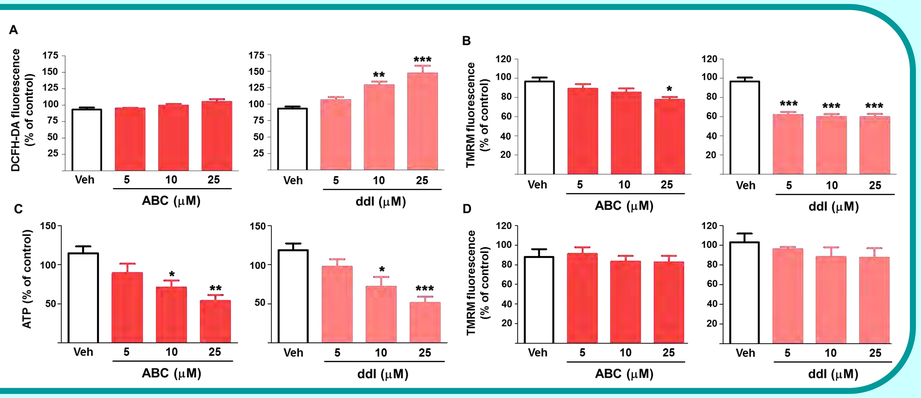


Fig.1: Effects of NRTI on O_2 consumption. Addition of NRTI to respiring Hep3B cells produced different effects according to the type of drug involved. Only ABC and ddI inhibited mitochondrial respiration at clinically relevant concentrations (indicated by an arrow).

Fig.2: Effects of ABC and ddI on mitocondria. 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)



APAP induces toxicity in Hep3B at high concentrations

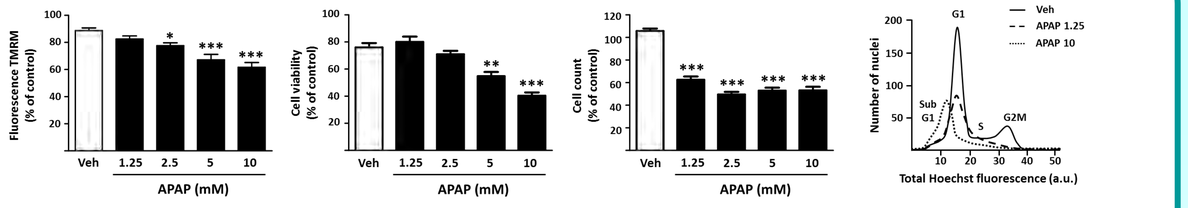


Fig.3: Mitochondrial and cellular toxicity of APAP. APAP exerted a cytotoxic effect in Hep3B at high concentrations of the drug (2.5-10 mM), while 1.25 mM induced low levels of toxicity, decreasing cell proliferation but not compromising cellular viability or mitochondrial function.

ABC and ddI increase vulnerability to APAP-induced hepatotoxicity

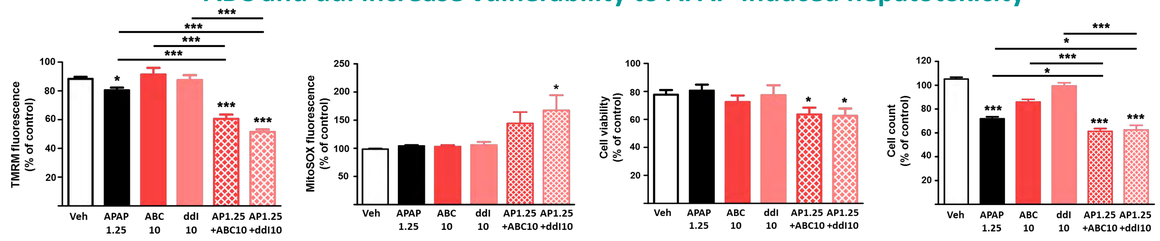


Fig.4: Analysis of mitochondrial function and cellular viability after co-administration of APAP. Co-administration of both stimuli (APAP and either ABC or ddI) enhanced the individual effects of each drug on mitochondrial function, cell count and viability.

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.