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A complex toxicity response involving both mitochondrial dysfunction and ER stress in human hepatic cells treated with the antiretroviral drug Efavirenz

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Background. Efavirenz (EFV) is the most widely used NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitors) employed within the current multidrug therapy of HIV-1 infection. A substantial group of patients receiving EFV report hepatotoxicity, which may become a reason for discontinuation of the therapy. The cellular mechanisms of this effect are still not elucidated. We have recently reported that short-term treatment (24h) of human hepatic cells with EFV undermines mitochondrial function, as manifested by compromised mitochondrial respiration, reduction in the mitochondrial membrane potential and an increase in mitochondrial reactive oxygen species generation.

Aim. To further analyze the mitotoxic capacity of EFV and explore additional pathway of specific cellular alterations related to the mitotoxicity induced by this drug

Methods. The experimental models involved the human hepatoma cell line Hep3B (ATCC HB-8064) and primary human hepatocytes, and treatments were performed for 8-24h with clinically relevant concentrations of EFV (10, 25 and 50□M) dissolved in methanol. Data were reported as mean+/-SEM, and statistical significance vs vehicle was analyzed by One-way ANOVA.

Results. We describe that EFV produces severe mitochondrial damage and induces mitophagy in human hepatic cells. Transmission electron microscopy revealed a significant reduction of the number of mitochondria, which were bigger in size and displayed deranged morphology with aberrant cristae. We also detected autophagic degradation of these organelles (mitophagy), evidenced by mitochondria-containing double-membrane vacuoles. Autophagy was also confirmed by Western blot studies of specific marker proteins: LC3 (light chain of the microtubule-associated protein) and Beclin-1. The severe mitochondrial morphology derangement was also observed with fluorescence microscopy (mitochondria stained NAO), an effect accompanied by enhanced mitochondrial mass as confirmed by western blot analysis. Interestingly, despite this increase, we detected an absence of *de novo* mitochondrial biogenesis (quantitative PCR). These mitochondrial alterations were paralleled by changes in the ER (Endoplasmatic Reticulum) function revealing the presence of ER stress in Hep3B cells exposed to EFV for 24h. Thus, a concentration-dependent increase in the mRNA of two ER stress markers, CHOP (CCAAT/enhancer binding protein) and GRP78 (Glucose-regulated protein 78), was detected by quantitative RT-PCR. Moreover, the protein levels of CHOP and GRP78 were enhanced, as well as the phosphorylation of eIF2□ (eukaryotic initiation factor 2). In addition, we also detected a significant increase in the cytosolic calcium content and in the presence of the specific ER signal studied by fluorescence microscopy employing FLUO-4AM and Lysotracker respectively.

Conclusion. Short-term treatment of hepatic cells with clinically relevant concentrations of Efavirenz, a member of the NNRTI family of antiretroviral drugs, produces a complex concentration-dependent toxic effect involving both alterations of the mitochondrial integrity and function as well as presence of ER stress. These cellular modifications may be relevant for better understanding of the clinical manifestations regarding liver hepatotoxicity in patients undergoing EFV-containing therapy.