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Poster presentation



# Clinical concentrations of efavirenz (EFV) reduce cellular proliferation and viability in several human cell lines

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## Background

Efavirenz (EFV)-containing therapies have been related to several side-effects including hepatotoxic events and chronic disorders in the lipid metabolism but the possible cellular mechanisms underlying these effects have received little study.

## Methods

In this work, we evaluated the cytotoxic effects of clinical (10–25 μM) and supraclinical (50 μM) concentrations of EFV in various human cellular models.

## Results

MTT assays upon 24 h of culture in the presence of the drug revealed reduced viability in the human hepatoma cell line Hep3B (significant for all three concentrations and calculated as 84.59 ± 8.82% decrease for 50 μM EFV), human cervix carcinoma cell line HeLa (71.92 ± 5.49% reduction for 50 μM EFV) and primary Human Umbilical Vein Endothelial cells (HUVEC), (96.76 ± 0.27% reduction for 50 μM EFV). This result was corroborated with 3-day-proliferation experiments in which Hep3B were exposed to different concentrations of EFV; a significant reduction (60.1 ± 6.54% after 3 days) was detected with 25 μM EFV whereas cytotoxicity (97.01 ± 1.13% reduction) was observed with 50 μM, however no changes were detected with 10 μM EFV. With the aim of analyzing the mechanisms responsible for this diminished cellular viability, we performed bivariate Annexin V/Propidium Iodide analysis of HeLa cells using static cytometry, and found that EFV-treated cells (4 and 8 h), presented features of late or advanced apoptosis. We also observed a dose-dependent translocation of two mitochondrial proapoptotic proteins, cytochrome c and AIF, in Hep3B cells after EFV-treatment (4 h), which was accompanied by a significant reduction in the mitochondrial membrane potential ($\Delta\psi m$), as measured by TMRM fluorescence. Confocal fluorescence microscopy experiments revealed a dose-dependent activation of caspase-3 and -9 and an absence of activation of caspase-8, pointing to EFV induction of the intrinsic (mitochondrial) apoptotic pathway.

## Conclusion

In conclusion, clinical concentrations of EFV can be cytotoxic and lead to activation of apoptotic programmes in common cellular models. This suggests that the therapeutic range of EFV is rather narrow and also that prolonged administration of this drug may result in HAART-related mitochondrial dysfunction.

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