EFAVIRENZ-INDUCED NITRIC OXIDE AFFECTS MITOCHONDRIAL FUNCTION IN GLIAL CELLS

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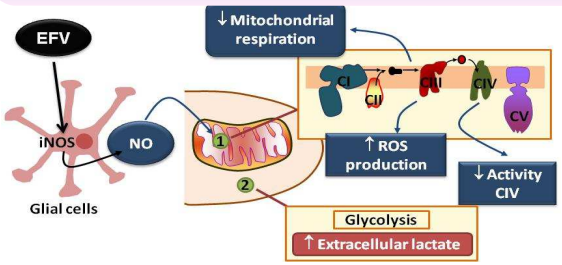
INTRODUCTION: Nitric oxide (NO) is a ubiquitous central nervous system (CNS) mediator implicated in both mitochondrial dysfunction and inflammation, and closely linked to neurological pathogenesis including that observed in HIV patients. More than 50% of Efavirenz (EFV)-treated patients exhibit CNS-related effects that often require discontinuation of the therapy[1]; moreover this drug has been recently linked to the development of HIV-associated neurological disorder (HAND). The underlying mechanisms of these effects are still unknown however, growing recent evidence, both *in vivo* and *in vitro*, points to mitochondrial dysfunction and altered CNS bioenergetics[2,3,4].

AIM: Using *an in vitro* approach, we analysed the ability of EFV to regulate NO generation in cultured neurons and glial cells and assessed the involvement of NO in the mitochondrial and bioenergetic action of EFV previously described in this model.

METHODS: Human cell lines glioma (U-251MG) and neuroblastoma (SH-SY5Y), and primary cultures of rat cortical neurons and astrocytes were exposed to short-term treatment with clinically relevant concentrations of EFV in plasma (10 and 25µM).

STATISTICAL ANALYSIS: Data are represented as % of control (considered 100%) and were analyzed using GraphPad Prism v.5 software by a one-way ANOVA multiple comparison test followed by Newman–Keuls test. All values are mean±S.E.M, n ≥3 and statistical significance was: *p<0.05, **p<0.01 and ***p<0.001 EFV vs Vehicle. Significance EFV vs co-treatment of DETA-NO+EFV was assessed by Student t-test ($^{+}$p<0.05, $^{++}$p<0.01 $^{+++}$p<0.001). Significance of the positive control condition was independently assessed vs untreated cells by Student t text ($^{\#}$p<0.05, $^{\#\#}$p<0.01 $^{\#\#\#}$ p<0.001).

CONCLUSION: EFV induces the synthesis of NO in glial cells which interferes with mitochondrial function and bioenergetic alterations in these cells, an effect not observed in neurons. These findings shed light on the mechanisms of the CNS side-effects of this drug, including the neuropsychiatric symptoms that appear soon after initiation of EFV therapy, which are sometimes accompanied by neuroinflammation, and possibly the long-term effects including HAND.



EFV-induced NO participates in:
(1) the mitochondrial actions
(2) the bioenergetic effect (up-regulation of glycolysis) triggered by EFV

REFERENCES: [1]**Abers MS, *et al.*, CNS Drugs 2014;28(2):131-45.** [2]**Streck EL, *et al.*, Neurochem Res 2011; 36(6):962-966.** [3]**Brown LA *et al.*, PLoS One 2014; 9(4):e95500.** [4]**Funes HA, *et al.*, J Infect Dis. 2014;210(9):1385-95.**

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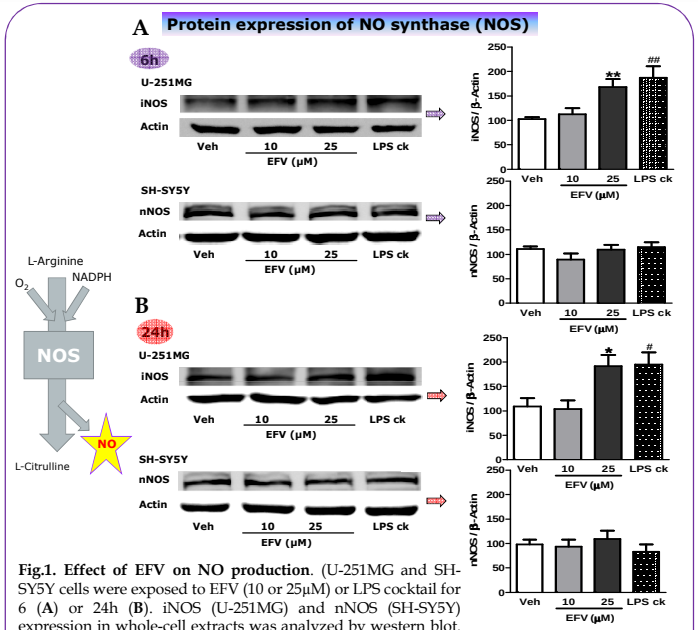

Fig.1. Effect of EFV on NO production. (U-251MG and SH-SY5Y cells were exposed to EFV (10 or 25µM) or LPS cocktail for 6 (**A**) or 24h (**B**). iNOS (U-251MG) and nNOS (SH-SY5Y) expression in whole-cell extracts was analyzed by western blot. Representative images and summary of the densitometry results (normalized to β–Actin expression) are shown.

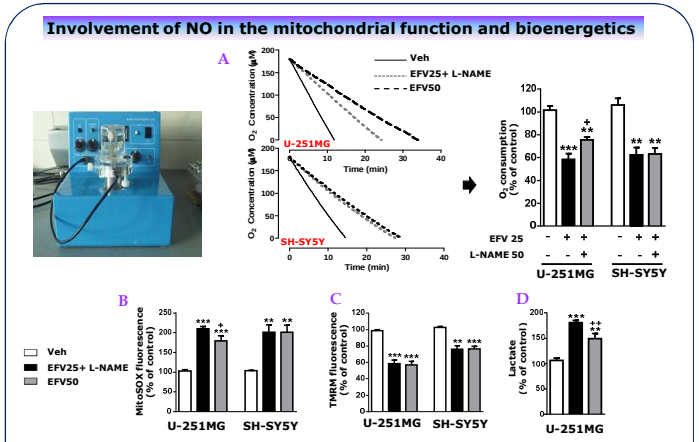

Fig.3. Interference of NO with mitochondrial function and bioenergetics. U-251MG and SH-SY5Y cells and were exposed to 25µM EFV or 25µM EFV + 50µM L-NAME. (A) O_2 consumption in intact cells (Clark-type O_2 electrode) after 6h. (B) Superoxide production (MitoSOX fluorescence), (C) mitochondrial membrane potential (TMRM fluorescence) and (D) extracellular lactate levels (absorbance) after 24h-treatment.

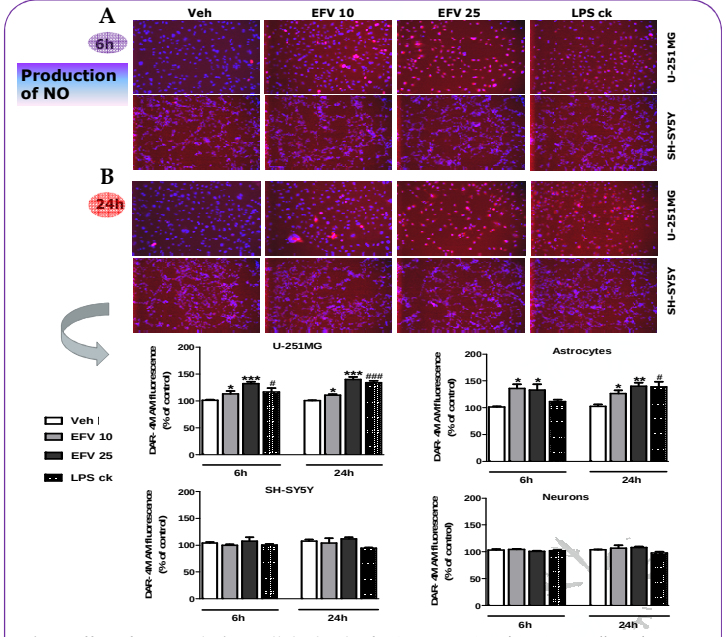

Fig 2. Effect of EFV on the intracellular levels of NO. U-251MG and SH-SY5Y cells and primary cultures of rat astrocytes and neurons were exposed to EFV (10 or 25µM) or LPS cocktail for 6 (**A**) or24h (**B**) and the intracellular NO content was assessed by fluorescence microscopy using the fluorochrome DAR-4M AM. Representative life-cell images (10x) showing nuclei (blue, Hoechst 33342) and NO (red signal), and quantification of the mean fluorescence is represented.

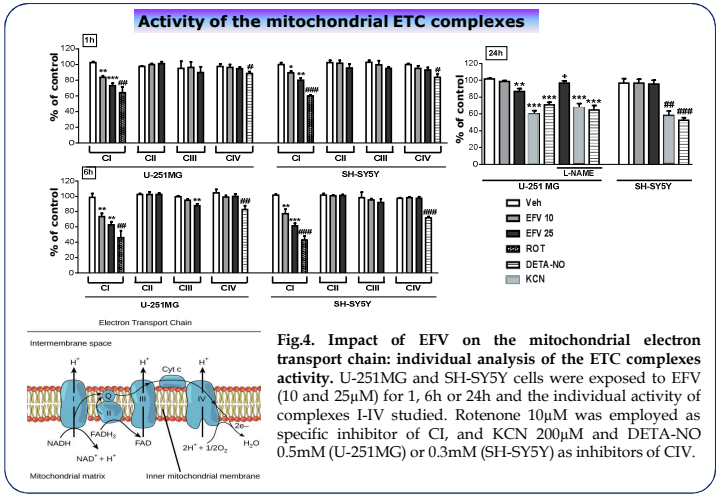

Fig.4. Impact of EFV on the mitochondrial electron transport chain: individual analysis of the ETC complexes activity. U-251MG and SH-SY5Y cells were exposed to EFV (10 and 25µM) for 1, 6h or 24h and the individual activity of complexes I-IV studied. Rotenone 10µM was employed as specific inhibitor of CI, and KCN 200µM and DETA-NO 0.5mM (U-251MG) or 0.3mM (SH-SY5Y) as inhibitors of CIV.