New insights in dose-dependent mechanisms involved in cyclosporine A-induced cholestasis in HepaRG cells. Comparison with tacrolimus

Ahmad Sharanek [1,2], Pamela Bachour-El Azzi [1,2], Houssein Al-Attrache [1,2], Camille C. Savary[1,2], Christiane Guguen-Guillouzo[1,2,3] and André Guillouzo [1,2]

[1] Inserm UMR991, Liver Metabolisms and Cancer, Rennes, France; [2] University of Rennes 1, Rennes, France; and [3] Biopredic International, St Grégoire, France. *Ahmad.Charanek@univ-rennes1.fr*

INTRODUCTION

Cyclosporine A (CsA) is a powerful immunosuppressant drug widely used in transplantation procedures and in the treatment of several autoimmune diseases. However, its therapy is associated with numerous side-effects, especially dose-related cholestasis. Mechanism(s) underlying these effects remain(s) largely unknown. Tacrolimus (FK506), a macrolide immunosuppressant that possesses similar but more potent (10-100 folds) immunosuppressant properties compared to CsA, is considered as an alternative primary immunosuppressant to CsA in hepatic transplantation. The effect of FK506 on bile flow is at the moment unclear and little is known about its effect at the canalicular level. The aim of the present work was to perform a comparative study of CsA and FK506 on canalicular function using the well differentiated human HepaRG cell line, considering the great difference in the therapeutic doses of both drugs.

RESULTS

CsA inhibits canalicular efflux more potently than FK506

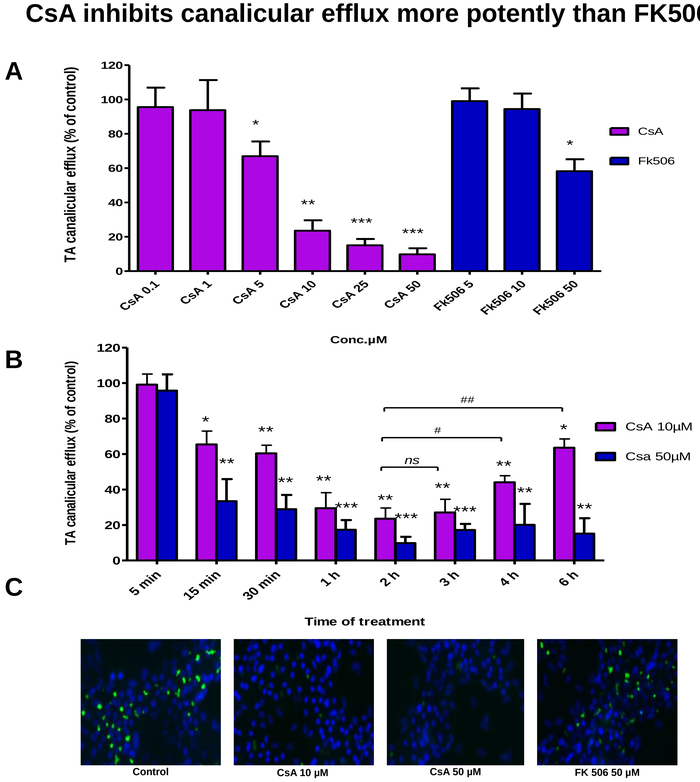


Figure 1 **HepaRG Cells** were exposed to [^{3}H]- taurocholic acid (TA) for 30 minutes then incubated (A) with different CsA or FK506 concentrations for 2 hours or (B) for different time points. TA efflux was determined by measuring intracellular TA accumulation. (C) MRP2 activity was estimated by the fluorescent substrate CDF.

RESULTS

Generation of ROS and ER stress by CsA

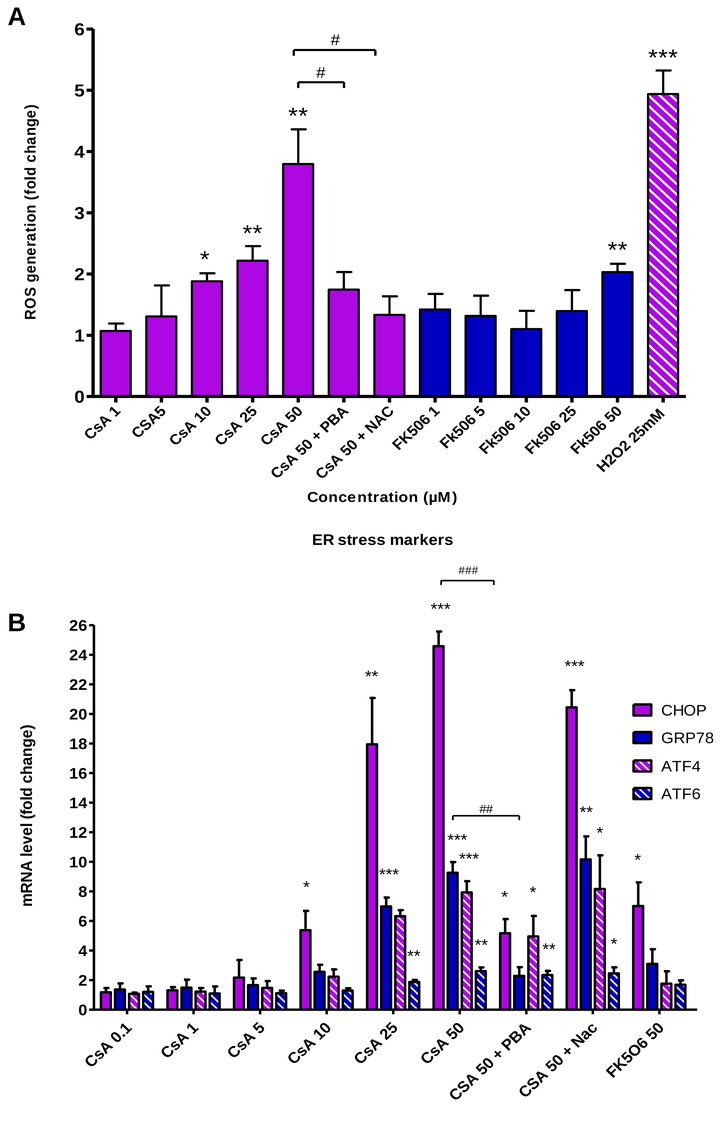


Figure 2 Cells were treated with either 50µM CsA alone, co-treated with N-acetyl-cysteine (NAC) 15 mM or 4-phenyl butyric acid (PBA) 6 mM, (A) ROS generation measured using the DCFDA fluorescent substrate. (B) mRNA levels of Endoplasmic reticulum (ER) stress markers were estimated by RT-qPCR analysis.

RESULTS

Role of cPKC, ER stress and ROS in CsA-induced cholestasis

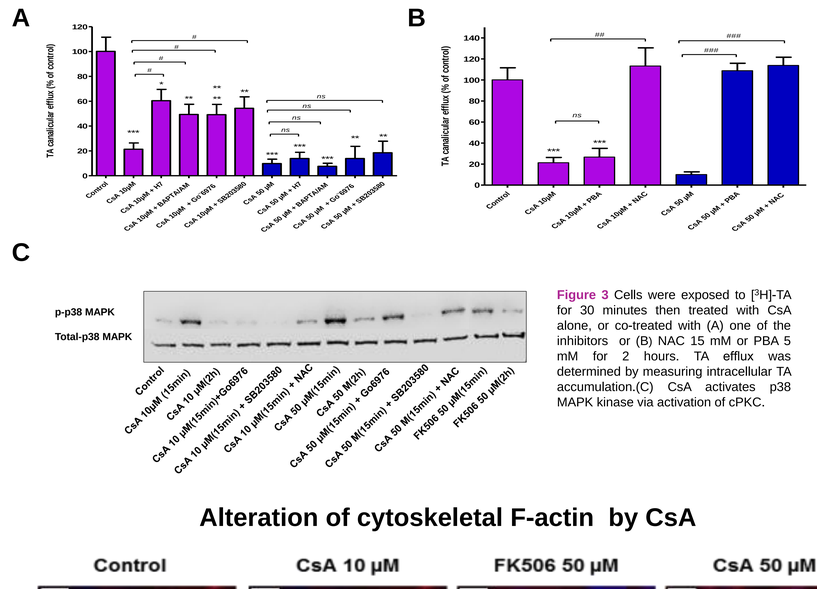
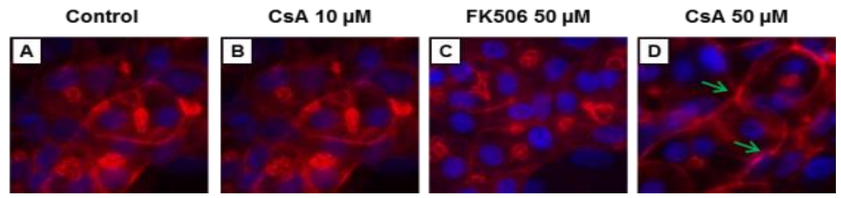


Figure 3 Cells were exposed to [^{3}H]-TA for 30 minutes then treated with CsA alone, or co-treated with (A) one of the inhibitors or (B) NAC 15 mM or PBA 5 mM for 2 hours. TA efflux was determined by measuring intracellular TA accumulation.(C) CsA activates p38 MAPK kinase via activation of cPKC.

Alteration of cytoskeletal F-actin by CsA



SUMMARY

- **CSA effects were dose-dependent:**
  - At low concentrations (<10µM) efflux inhibition was reversible and was associated with changes in cPKc signaling and induction of an oxidative stress
  - At high concentrations (50 µM and higher) efflux inhibition was irreversible and was associated with an endoplasmic reticulum stress induction and alteration of cytoskeletal F-actin.
- **FK506** did not induce obvious cholestatic features even at high concentrations (it is 10 to 100-fold more potent than CsA as an immunosuppressant agent and is used at 10 to 100-fold lower concentrations in vivo
- **CPZ** (idiosyncratic drug) induces cholestasis only at high concentrations (Antherieu et al. Hepatology, 2013)
  - Since Cmax values measured in patients are 1.15, 0.1-0.2 and 0.05 µM for CsA, CPZ and FK506 respectively, cholestatic concentrations are expected to be reached only in patients treated with CsA.
  - These findings suggest that it could be possible to discriminate between dose-dependent and idiosyncratic cholestatic drugs using the HepaRG cell model.

  (Manuscript in preparation: Sharanek et al.)