Alterations of the Bile Acid transport system by Cyclosporine A in HepaRG cells

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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 FK506 and CsA 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

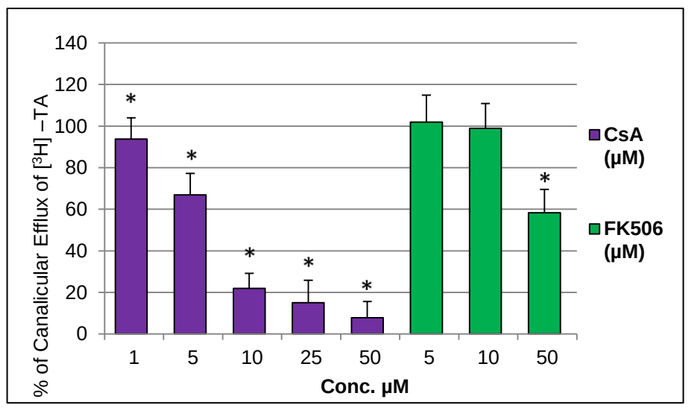


Figure 1 **HepaRG Cells** were exposed to [3H]- taurocholic acid (TA) for 30 minutes then incubated 2 hours with different CsA or FK506 concentrations. TA efflux was determined by measuring intracellular TA accumulation. arbitrarily set at a value of 100%. Data represent the means ± SEM

Effect of FK506 and CsA on NTCP

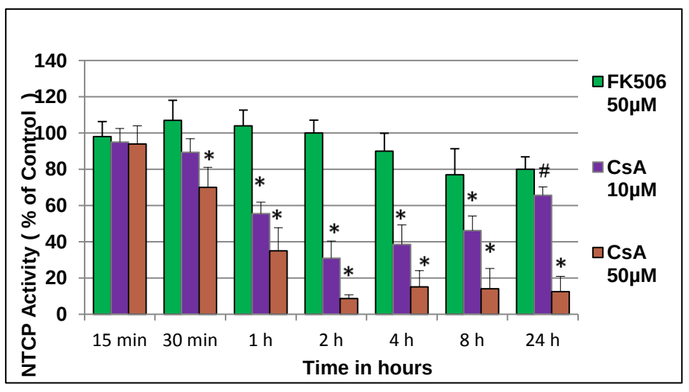


Figure 2 **HepaRG cells** were treated with CsA or FK506 for different time points, then exposed to [3H]- taurocholic acid (TA) for 30 minutes then incubated. TA influx was determined by measuring intracellular TA accumulation. arbitrarily set at a value of 100%. Data represent the means ± SEM.

RESULTS

Involvement of cPKC-P38 in CsA induced cholestasis

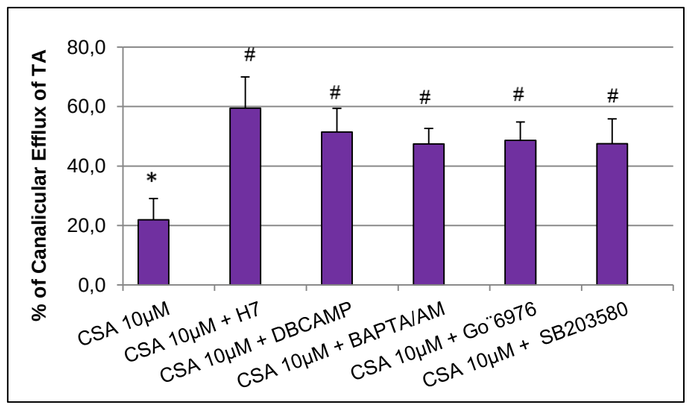


Figure 3 **Fig 3: HepaRG Cells** were exposed to [3H]- taurocholic acid (TA) for 30 minutes then incubated 2 hours with either CsA 10µM or CsA plus one of the inhibitors in the table below.*P < 0.05 compared with control, # P < 0.05 compared with CsA 10µM alone

H7	Inhibitor of PKC
BAPTA/AM	Ca2+ chelator
Go6976	cPKC inhibitor
SB203580	P38 inhibtor
DBcAMP	Activator of PKA

RESULTS

Generation of ROS and ER stress by CsA at high dose

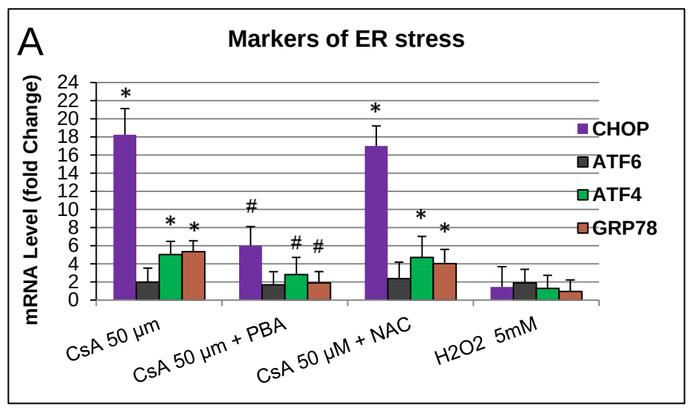


Figure 4 Cells were treated with either 50µM CsA alone, co-treated with N-acetyl-cysteine (NAC) 15mM or 4-phenyl butyric acid (PBA) 6mM for 6 hours. mRNA levels of (A) ROS markers and (B) Endoplasmic reticulum (ER) stress markers were estimated by RT-qPCR analysis. (C) ROS generation measured using the DCFDA fluorescent substrate.*P < 0.05 compared with control, # P < 0.05 compared with CsA 50µM alone.

RESULTS

Involvement of ER stress and ROS in CsA-induced cholestasis at high dose

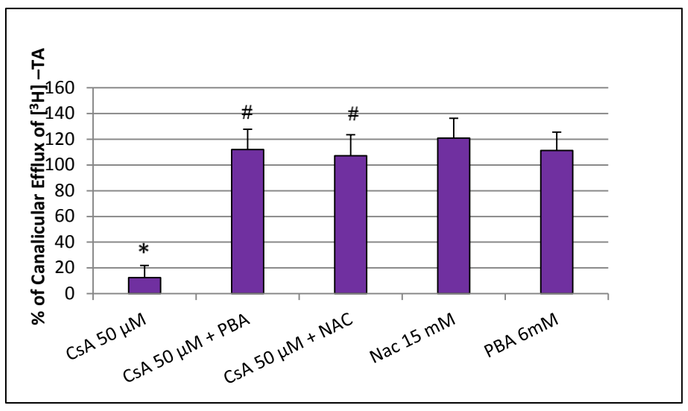


Figure 5 Cells were exposed to [3H]-TA for 30 minutes then treated with 50µM CsA alone, co-treated with NAC 15mM or PBA 6mM for 2 hours . TA efflux was determined by measuring intracellular TA accumulation. Data were expressed relative to the level found in control cells. Control arbitrarily set at the value of 100%.*P < 0.05 compared with control, # P < 0.05 compared with CsA 50µM alone.

Alteration of rearrangement of the F-actin cytoskeleton by CsA treatment

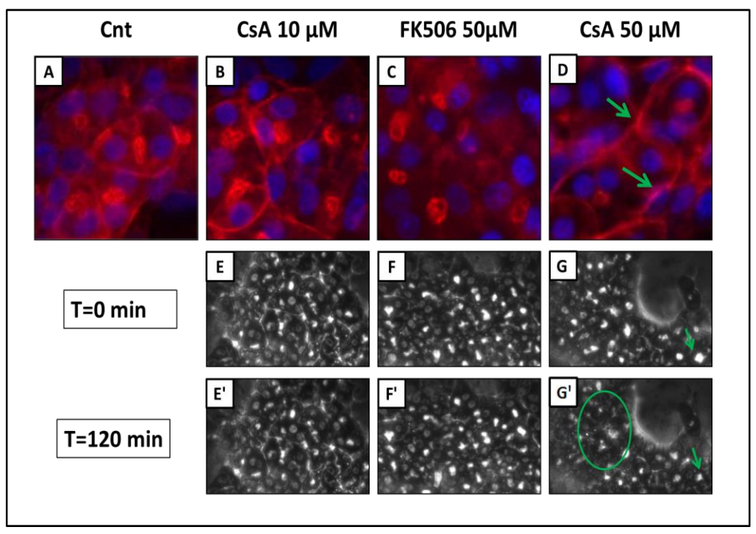
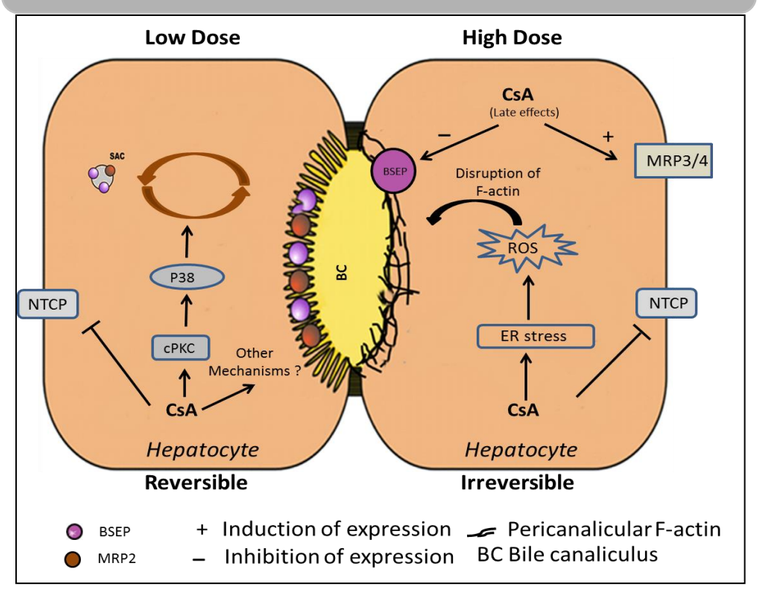


Figure 6 (A) Control. (B) Cells treated with CsA 10 µM. (C) Cells treated with FK506 50µM (D) Cells treated with CsA 50 µM. F-actin was localized by using phalloidin-flouroprobe. Nuclei were stained in blue (Hoechst). F-actin shows a predominant pericanalicular distribution in untreated cells and a less intense staining around the canalicular region in 50 µM CsA-treated cells. Time-lapse imaging of HepaRG cells treated with (E') CsA 10 µM after 120 min (F') FK506 50 µM after 120 min and (G') CsA 50 µM after 120µM compared to theirT0 time:(E), (F) and (G) respectively.

SUMMARY



- **Ca^{2+} dependent PKC-P38 is involved in CsA induced cholestasis at low doses, however at high doses CsA induces ROS generation and disruption of pericanalicular F-actin distribution.**
- **Our results support the conclusion that FK506 is not cholestatic at therapeutical doses**