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IDENTIFICATION AND IN VITRO CHARACTERIZATION OF A NOVEL BSEP AND ABCB4 DUAL-ACTING POSITIVE FUNCTIONAL MODULATOR TARGETING THE TREATMENT OF A BROAD RANGE OF HEPATOBILIARY DISEASES

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RECTIFY PHARMA

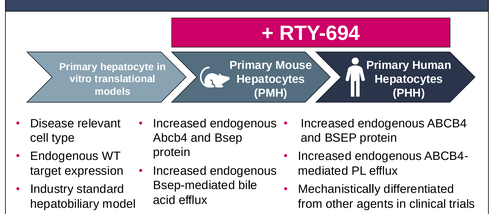
Introduction

In hepatocytes, bile salts secreted by BSEP (ABCB11) are complexed with cholesterol and the phospholipids transported by MDR3 (ABCB4) into the canalicular space (1, 2). These mixed micelles protect the biliary epithelium from the detergent properties of bile salts and the pro-inflammatory effect of free cholesterol, and facilitate lipid digestion. The precise composition of these biliary micelles is tightly regulated by these transporters to prevent hepatobiliary injury (3, 4).

Dysregulation of bile homeostasis is associated with a spectrum of liver diseases (5). Inherited homozygous loss-of-function mutations in BSEP and ABCB4 lead to progressive familial intrahepatic cholestasis (PFIC) type 2 and type 3, respectively (8, 9). Additionally, disruption of bile acid equilibrium and excretion contributes to secondary cholestatic diseases such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) (10).

RTY-694 is a small molecule that acts as a dual-targeted positive functional modulator that selectively enhances the protein levels and function of BSEP and ABCB4. Therapeutic strategies that increase BSEP and ABCB4 function in a coordinated fashion in hepatocytes could potentially improve outcomes in a variety of hepatobiliary diseases.

Overview



Methods

Freshly isolated primary mouse hepatocytes (PMH) were obtained from MB Biosciences (Natick, MA). Cryo-preserved and freshly isolated primary human hepatocytes (PHH) were obtained from Invitrogen and BioIVT (Baltimore, MD), respectively. PMH and PHH cells were cultured in 24-well or 96-well plate formats for the indicated durations, and vehicle or compounds were added to the culture medium at the indicated concentrations. The levels of BSEP and ABCB4 protein, functional activity, and mRNA were assessed in response to the different treatments and compared to vehicle control.

RTY-694 Increases Transporter Levels and Bile Acid Efflux in Primary Mouse Hepatocytes

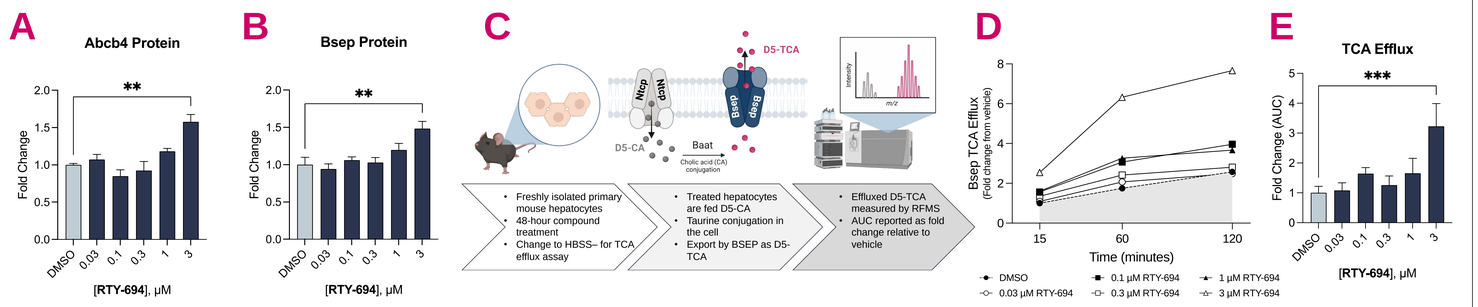


FIGURE 1. RTY-694 increases endogenous Abcb4 and Bsep protein and Bsep function. Densitometric quantitation of **(A)** Abcb4 and **(B)** Bsep immunoblot following RTY-694 treatment of PMHs for 48 hours normalized to the beta-actin loading control. **(C)** Primary mouse hepatocyte taurocholate efflux assay method. **(D)** D5-taurocholic acid (TCA) efflux in PMH treated with RTY-694 normalized to the 15-minute time point for the vehicle treated samples. Symbols represent mean value of n = 3 independent replicates. **(E)** Integrated AUC for the 120-minute time course of D5-TCA in PMH treated with RTY-694. Statistical significance relative to vehicle control was determined by ANOVA; * p < 0.05, ** = p < 0.01, *** p < 0.005.

RTY-694 does not act via transcription

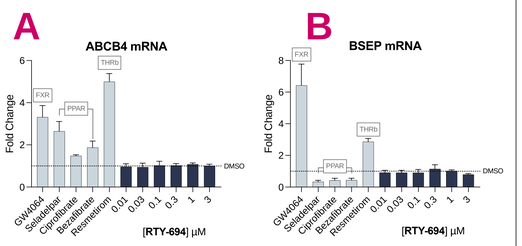


FIGURE 3. RTY-694 does not significantly impact mRNA levels of ABCB4 or BSEP in primary human hepatocytes. (A) ABCB4 and **(B)** BSEP mRNA levels in primary human hepatocytes treated with DMSO, 10 μM test compound (GW0464, Seladelpar, Ciprofibrate, Bezafibrate, Resmetirom), or RTY-694 at the indicated concentrations for 24 hours normalized to GAPDH expression and relative to vehicle (DMSO)-treated control.

RTY-694 Increases Transporter Levels and Phospholipid Efflux in Primary Human Hepatocytes

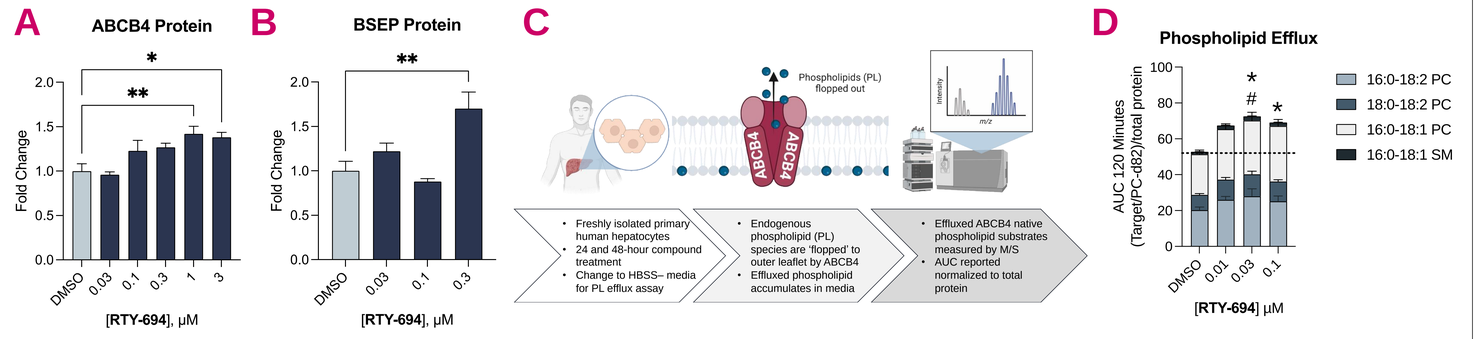


FIGURE 2. RTY-694 increases endogenous ABCB4 and BSEP protein and ABCB4 function. Densitometric quantitation of **(A)** ABCB4 and **(B)** BSEP immunoblot following RTY-694 treatment of PHHs for 24 hours normalized to the beta-actin loading control. Statistical significance relative to vehicle control was determined by ANOVA; * p < 0.05, ** = p < 0.01. **(C)** Primary human hepatocyte phospholipid efflux assay method. **(D)** ABCB4-mediated phospholipid species efflux in primary human hepatocyte cells treated with RTY-694 at different concentrations and normalized to internal standard and cellular protein. Statistical significance relative to vehicle control was determined by ANOVA; * p < 0.05 for 16:0-18:1 PC, # p < 0.05 for 16:0-18:2 PC versus DMSO. Dotted line indicates total effluxed phospholipid in DMSO-treated samples.

Conclusions

In primary hepatocytes, **RTY-694** directly and selectively increases both BSEP and ABCB4 protein levels and the cellular efflux of bile acids and phospholipids mediated by these ABC transporters. This mechanism is distinct from other small molecules that act at the gene expression level. As a novel dual-acting BSEP and ABCB4 PFM, **RTY-694** has the potential to provide benefit to patients with a broad range of hepatobiliary diseases where dysregulated BSEP and ABCB4 activity leads to cholestasis and cholangitis.

References

1. Gerloff T, et al. (1998). J Biol Chem. PMID: 9545351.
2. Oude Elferink RP, et al. (1995). J Clin Invest. PMID: 7814632.
3. Boyer JL. (2013). Compr Physiol. PMID: 23897680.
4. Hofmann AF. (2009). Hepatology. PMID: 19296471.
5. Nayagam JS, et al. (2020). Aliment Pharmacol Ther. PMID: 33070363.
6. Rosmorduc O, et al. (2001). Gastroenterology. PMID: 11313316.
7. Pauli-Magnus C, et al. (2004). Pharmacogenetics. PMID: 15077010.
8. Strautnieks SS, et al. (1998). Nat Genet. PMID: 9806540.
9. de Vree JM, et al. (1998). Proc Natl Acad Sci U S A. PMID: 9419367.
10.Aseem SO, et al. (2023). Cells. PMID: 36899928.