

1. INTRODUCTION

Hepatic lipogenesis catalyzed by fatty acid synthase (FASN), is a driver of inflammation and steatosis in NASH. We have tested the activity of a specific FASN inhibitor, TVB3664, in an established murine model of NASH characterized by advanced fibrosis and hepatocellular carcinoma (Tsuchida et al., 2018. J Hepatol, 69: 385-395), based in part on its antifibrotic effect in the human hepatic stellate LX-2 cell line (AASLD 2017 #1994).

2. AIMS

This study was to evaluate the impact of a small molecule of FASN inhibitor (TVB3664) synthesized by 3V Biosciences in a diet and CCl₄ induced mice NASH model that reproducibly induces significant fibrosis and hepatocellular carcinoma (HCC) which characteristically close to human NASH.

3. METHODS

- Six week-old male C57BL/6J mice were fed a high fat-cholesterol western diet (WD) and glucose-fructose sugar water (SW) combined with 0.2µl/gm body weight CCl₄ injection via IP (once/week) for 24 weeks (Tsuchida et al., 2018. J Hepatol, 69: 385-395),
- From week 13 to week 24, animals were additionally administered oral daily doses of either vehicle (30% PEG400 in water) or three different doses of TVB3664 (3 mg/kg, 5 mg/kg or 10 mg/kg in vehicle)
- After 24 weeks (12 weeks of treatment period) mice were sacrificed and necro-inflammatory activity, NASH activity, expression of profibrotic genes and protein were assessed

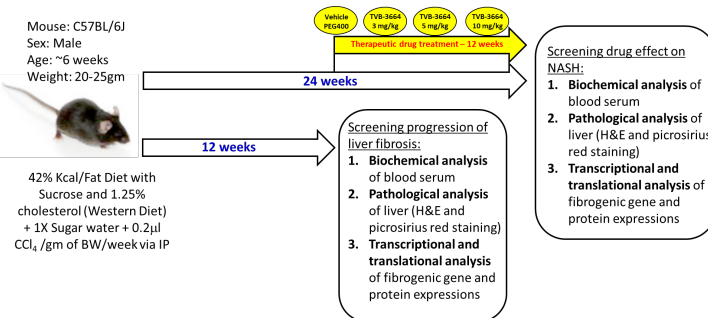


Figure 1: In vivo model of diet and CCl₄ induced NASH mice and treatment with TVB3664 small molecules

4. RESULTS

Necro-inflammatory activity is improved on TVB3664 treatment

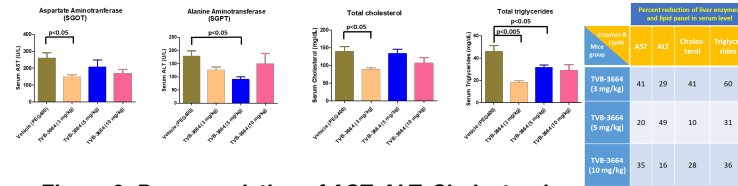


Figure 2: Downregulation of AST, ALT, Cholesterol and Triglycerides in serum by TVB3664

NAFLD activity is significantly improved on TVB3664 treatment

Histopathological analysis of liver sections

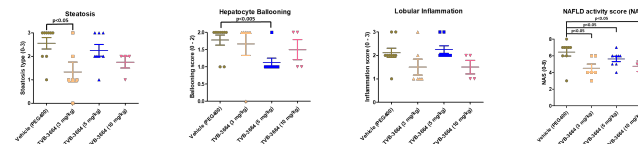


Figure 3: TVB3664 reduced steatosis, ballooning, inflammation and NAS in NASH mice

TVB3664 reduces hepatic collagen

Morphometric quantification of collagen by BIOQUANT image analysis software and Picrosirius red and fast green stained liver sections

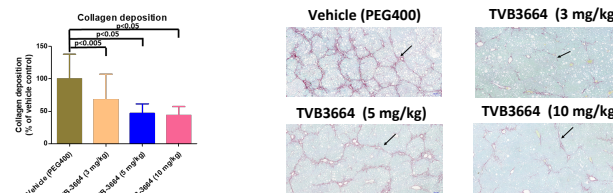


Figure 4: Dose dependent reduction of fibrillar collagen (black arrow) deposition in liver is observed on TVB3664 treatment

TVB3664 reduces profibrotic gene expression

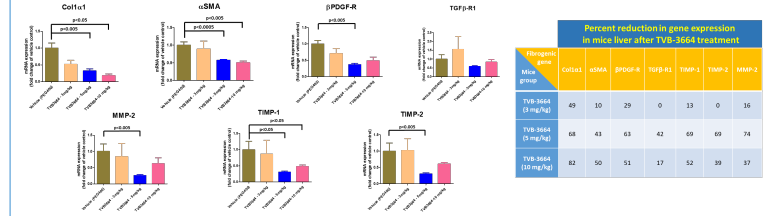


Figure 5: Relative mRNA expression of profibrotic gene/GAPDH in mice liver tissue was measured by RT-qPCR and fold change was calculated over vehicle control

TVB3664 reduces fibrogenic protein expression

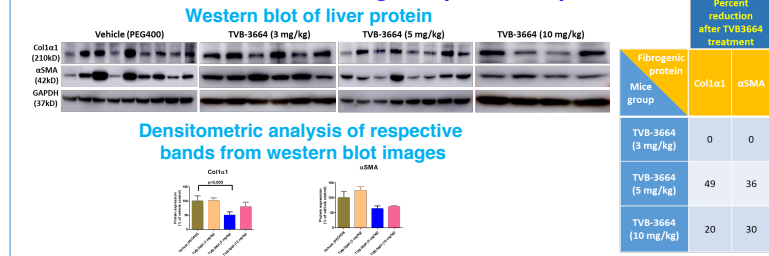


Figure 6: Relative expression of Collagen1a1 and αSMA protein were reduced in NASH mice liver on TVB3664 treatment

TVB3664 reduces tumor development

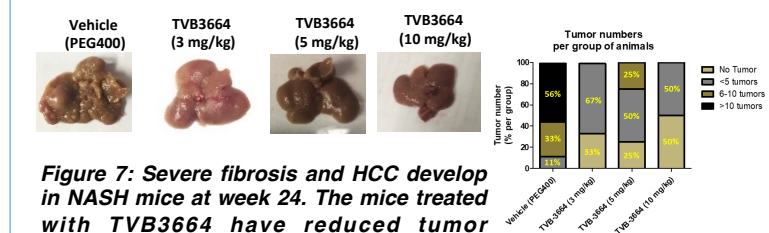


Figure 7: Severe fibrosis and HCC develop in NASH mice at week 24. The mice treated with TVB3664 have reduced tumor development.

CONCLUSIONS

- Biochemical analysis of liver injury (AST and ALT) and histopathological data including collagen deposition, NAS and fibrosis stage indicate that TVB3664 reduces NASH activity and associated fibrosis
- TVB3664 reduces the expression levels of all profibrotic genes as well as protein (Collagen1a1 and αSMA) in liver tissues
- Reduction of tumor numbers by TVB3664 indicates the potential therapeutic activity of TVB3664
- These results indicate that the **Fatty Acid Synthase inhibitor (FASNI) TVB3664 may be a potential candidate for further investigation as an antifibrotic drug in NASH**