AXL: a potential target to alleviate Tetracycline-induced liver steatosis.

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Introduction

- ✓Drug-induced steatosis is a side effect associated with various drugs arising from the excessive accumulation of fats in liver cells. This effect can interfere with normal liver function and progress into more severe anomalies (1).
- ✓Specific drugs causing this problem include Tetracycline, Valproic Acid, Amiodarone, Methotrexate etc. (2).
- ✓Receptor tyrosine kinases (RTKs) play critical for various cellular processes and cell types including hepatocytes. Thus, it is worthwhile to investigate whether particular RTK plays a crucial role in regulating lipid metabolism in hepatocytes, making them potential therapeutic targets for treating drug-induced steatosis.
- ✓Based on primary screening, we identify that AXL inhibition alleviates steatosis in hepatocyte cell lines.

Objective

- ✓To study effect of AXL inhibitors on tetracycline induced steatosis and its underlying pathway

Materials and Methods

- ✓**HepG2** cells were pre-treated with **Oleic Acid** (0.075mM) and **Tetracycline** (100µM) to mimic drug-induced steatosis.
- ✓Effect of AXL on steatosis was investigated using AXL inhibitor, Bemcentinib at concentration 5 µM along with Oleic acid and Tetracycline.
- ✓**BODIPY 493/505** fluorescence was measured by **flow cytometry** for quantitative analysis of lipid accumulation. **Confocal microscopy** was used for qualitative analysis of lipid accumulation.
- ✓**RT-PCR** was done to quantify relative mRNA expression of genes involved in lipid metabolism such as SREBP1c, FASN, CD36, PPARα and CPT1 normalized to GAPDH
- ✓**Immunoblotting assays** were performed to observe changes on protein expression of transcriptional factors such as SREBP1c and PPARα. Changes in activation of mTOR and ERK pathway were analysed by immunoblotting assay. All samples were normalised to GAPDH

Results

Bemcentinib reduces lipid accumulation in hepatocytes such as HepG2 cells and L O2 cells

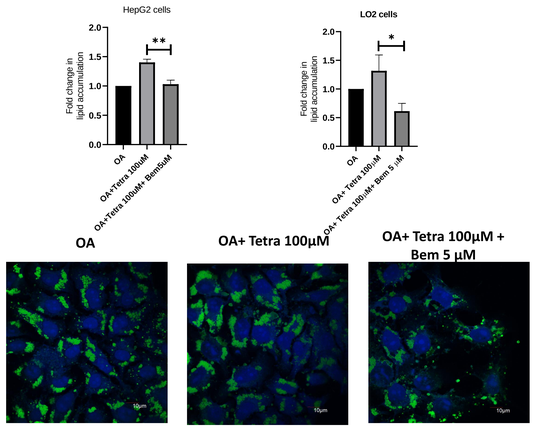

Fig.1 Effect of AXL inhibition on lipid accumulationA. Effect of Bemcentinib on tetracycline induced lipid accumulation in HepG2 and L 02 cells. B. Confocal image of HepG2 cells treated with OA, OA with Tetra100µM, OA with Tetra100µM in presence of Bemcentinib 5 µM. OA: Oleic Acid, Tetra: Tetracycline, Bem: Bemcentinib. One –way ANOVA, n= at least 3, *p<0.05, ***p<0.005, ****p<0.0001

Bemcentinib inhibits de-novo Lipogenesis, Fatty acid uptake and induces β-oxidation

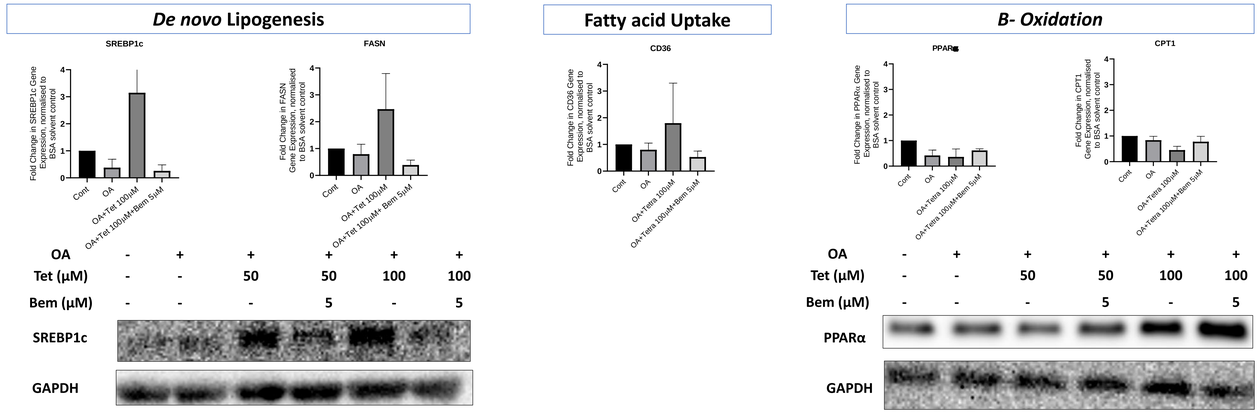

Fig. 2 Effect of AXL inhibition on lipid metabolism in HepG2 cells. A. Effect of Bemcentinib on genes involved in lipid metabolism was analysed using RT-PCR. Bemcentinib reduced gene expression of SREBP1c and FASN involved in *de-novo* lipogenesis. It also reduced expression of gene CD36 which is involved in fatty acid uptake. Bemcentinib, induced expression of genes involved in β-oxidation such as PPARα and CPT1. B. Effect of AXL inhibition on expression of transcriptional factors such as SREBP1c and PPARα normalized to GAPDH. OA: Oleic Acid, Tetra: Tetracycline, Bem: Bemcentinib. One –way ANOVA, n= at least 3 for SREBP1c, FASN and n=2 for CPT1, PPARα and CD36for RT-PCR. N=2 for immunoblotting analysis. *p<0.05, ***p<0.005, ****p<0.0001

Bemcentinib reduces activation of mTOR and ERK pathway which may regulate lipid metabolism

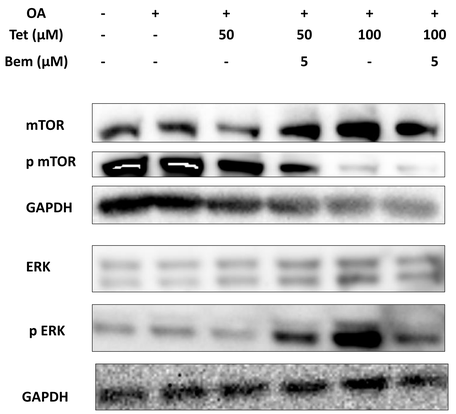

Fig. 3 Effect of AXL inhibition on mTOR and ERK pathway OA: Oleic Acid, Tetra: Tetracycline, Bem: Bemcentinib. One –way ANOVA. n=2 for immunoblotting analysis. *p<0.05, ***p<0.005, ****p<0.0001

Conclusion and Discussion

- ✓AXL inhibition alleviates drug induced steatosis and could be explored as therapeutic strategy to manage steatosis.
- ✓AXL inhibition alters the regulation of lipid metabolism. It reduces *de novo* lipogenesis and fatty acid uptake, while induces β-oxidation.
- ✓AXL inhibition modulates transcriptional factors such as SREBP1c and PPARα involved in *de novo* lipogenesis and β-oxidation via ERK and mTOR pathway.
- ✓This study provides a prototype approach to explore other RTKs for steatosis induced by various other drugs

Reference

(1) Cataldi M. *et.al.* Adv. Ther. 2021
(2) Kolaric, T. O.; Nincevic, V. and Kuna, L. et.al. J. Clin. Transl. Hepatol. 2021.

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