Detailed Analysis of the Antifibrotic effects of INT-767, a dual FXR/TGR5 agonist, in an obese mouse model of diet-induced and biopsy-confirmed NASH

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Introduction and Aim

The farnesoid X receptor (FXR) and G protein coupled receptor (GPR) TGR5 have been implicated in the regulation of metabolism, inflammation and fibrosis in non-alcoholic steatohepatitis (NASH). A dual FXR/TGR5 agonist, INT-767, has been developed and is in early clinical development. Here we evaluated the efficacy of INT-767 on qualitative and quantitative histopathological parameters in an obese mouse model of diet-induced and biopsy-confirmed NASH.

Methods

Male leptin-deficient Lep(ob)/ob mice [5 weeks of age] were fed a diet high in trans-fat, fructose and cholesterol (ob/ob-NASH). Livers were biopsied after 12 weeks on diet, and mice with steatosis scores 2-3 and fibrosis (stage 3-4) were randomized (n=10/group) to receive vehicle (PO, QD) or INT-767 (10 mg/kg, PO, QD) for 8 weeks. Primary endpoints included (i) blinded histological evaluation of fibrosis, total NASH Activity Score (NAS) and its components. Liver fibrosis was analyzed using picro-Siris Red (SR) staining and Col1a1 immunohistochemistry. Second harmonic generation (SHG) microscopy was used to quantify collagen fiber density (total brightness of collagen intensity) and collagen structure network (de-orientation, branch nodes). Lipid deposition was estimated by H&E staining and two-photon excitation fluorescence (2PE), with droplet size by 2PE. Immunohistochemical staining for fractional area (basement membrane remodeling), galectin-3 (macrophages) and alpha-SMA (activated hepatic stellate cells) was also assessed.

Study Design

Liver pre-biopsy procedures and in vivo efficacy

NASH histopathology and morphometry

- Morphometric analyses of liver fat area (morphometry) compared 2-PE analysis of fat area, and mean fat droplet size. INT-767 showed a strong effect on fat assessed by both methodologies. Fat droplets (white), altered structures (yellow), collagen (green), auto-fluorescence (blue).
- Data presented as mean x SD. **p<0.01, ***p<0.001 (unpaired t-test).

Quantification of collagen deposition

- Comparative image analyses of collagen content. Collagen content was determined by morphometric analyses of Col1a1 (immunohistochemistry and Sirius Red), and compared to SHG analysis of collagen fiber content and collagen fiber density. INT-767 reduced collagen content significantly and tended to reduced collagen fiber density.
- A clear correlation was observed across stainings and methodologies. Data are expressed as % of total parenchymal area fractionation of fat area. ***p<0.001 (unpaired t-test).

Conclusion

- INT-767 treatment improved liver histopathology in ob/ob-NASH mice by improving NAS and Fibrosis Stage.
- INT-767 decreased liver lipid load and reduced mean lipid droplet size.
- INT-767 decreased basal membrane fibrosis (laminin), inflammation (galectin-3) and activated hepatic stellate cells (alpha-SMA).
- These findings demonstrate the added value of using multiple quantitative imaging methodologies to complement NASH histological scores.